Information regarding other infections that do not require hospitalization will be provided in the annual reporting of the patient's clinical status.

9.6 SERIOUS ADVERSE EVENT REPORTING

9.6.1 Definition of a Serious Adverse Event

A serious adverse event is defined by the ICH GCP as any untoward adverse events that:

a. result in death,

b. are life-threatening,

c. require hospitalization (except as per previously mentioned),

d. cause persistent or significant disability/incapacity,

e. result in congenital anomalies or birth defects,

f. other conditions which in the judgment of the Investigators represent significant hazards.

g. Also included are any Grade 3 or Grade 4 toxicities as indicated in Appendix A (Toxicity Chart for Adverse Event Monitoring)

Examples include but are not limited to: any major deterioration of organ function (cardiac, renal, liver, pulmonary, hemopoietic) or mental status. This will be reported as a serious adverse event whether or not the event appears to be related to the gene transfer product (see Appendix A for Toxicity Chart for Adverse Event Monitoring).

9.6.2 General SAE Reporting Guidelines

Unless otherwise specified in this protocol and approved by the IRB, all serious adverse events will be reported to the IRB, IBC, OBA, NIAID's Clinical Director, and the FDA within 7 days for death or life threatening adverse events or 15 days for all others. All non-serious adverse events will be reported to the IRB during the annual continuing review, as described in Section 9.4.

As required by the FDA guidelines, any positive RCR result from patient monitoring will be reported immediately as an adverse event regardless of clinical outcome. Upon the death of any patient for any cause, an autopsy will be sought. Studies will be conducted to assess the presence of the therapeutic transgene or RCR in tissue samples that are available, as discussed at the end of Section 8.0 above.

10.0 DATA AND SAFETY MONITORING PLAN

The DSMB is an independent board including individuals with expertise in gene transfer methodology and clinical trial design, in inherited immune deficiencies and in biostatistics and bioethics. The DSMB is appointed by the Office of the Clinical Director, NIAID to order to monitor the conduct of this protocol, ensure the safety of participants and the validity and integrity of the data. After the initial DSMB review, which occurs prior to opening the study to enrollment, the DSMB will review cumulative study data to evaluate safety, study conduct, and scientific validity and integrity of the trial on a semi-annual basis. Regular reports of trial data will be prepared by the protocol team for review by the DSMB in regularly scheduled meetings. In addition, the DSMB and the Clinical Director will be notified of any Grade 3 or Grade 4 toxicity or other serious adverse event. DSMB members must be satisfied that the timeliness, completeness, and accuracy of the data submitted to them for review is sufficient for evaluation of the safety and welfare of study participants. All cumulative safety data reports from the trial will be submitted to the Board within ten business days prior to the review, which will take place twice a year.

All reports of trial data, Grade 3 and 4 adverse events, and other serious adverse events should be submitted to the DSMB Executive Secretary. The contact information for the DSMB Executive Secretary is:

Regulatory Compliance and Human Subjects Protection Program
SAIC Frederick, Inc.
5704 Industry Lane, Suite J
Frederick, MD 21702
FAX: 301-846-6224

The DSMB may make independent assessments of treatment safety/effectiveness and will provide a report of their findings to the Clinical Director and the IRB. The DSMB operates according to standard procedures approved by the Clinical Director. These procedures will comply with and will be revised according to:

a. NIH's policy requiring all gene transfer clinical trials sponsored by NIH to be monitored by a DSMB. (OHSR, NIH, Information sheet #18 "Guidelines for NIH Intramural Investigators and IRB on DSMB Monitoring").

b. Current GCP Standards, as described in FDA consolidated guidance on Good Clinical Practice, ICH E6.

c. FDA Guidance on Statistical Principles for Clinical Trials, ICH E9.

11.0 DATA MANAGEMENT PLAN

11.1 COLLECTION, MANAGEMENT, AND STORAGE OF RESEARCH DATA

Good Clinical Practice (GCP) mandates that clinical investigators prepare and maintain adequate and accurate case histories, observations, and other data pertinent to a particular research study and subject. These study records may include a variety of original information sources (i.e., source documents) from which data are obtained to complete the study's data collection forms (CRFs), or to corroborate the recorded data. Source documentation is composed of these original documents from which study data are abstracted. Some CRFs are well suited to have study data collected directly on them (e.g., physical examination form) and can serve as the source documentation. CRFs will be developed and provided for the patients by an independent Contract Research Organization. CRFs will be kept in a 3-ring notebook and divided by study visit. The patient identification number (PIN) will be assigned at the time of patient enrollment by the PI or study coordinator. Forms linking the PIN with the patient's name will be kept in a secure area that is separate from the completed data forms to ensure confidentiality. CRFs will be completed and maintained by staff on a designated list. This list will be kept in the regulatory binder.

The subject will be required to return to the Clinical Center within +/- 6 weeks of months 1, 2, 3, 6, 12, 18, 24, 30, 36, 42 and every 6-12 months thereafter for a minimum of 15 years per FDA Industry Guidances governing participants in gene therapy studies. Due to the chronic nature of the disease, the distance, and travel needs, the patient may miss a scheduled study visit. Should this occur, every effort will be made to re-schedule the missed visit within the allotted time period (6 weeks) or, if not possible, to collect interview health history data via phone from the patient, and/or his local medical care provider. Additionally, if a visit is missed, a blood collection kit will be sent to the subject to have his local medical care provider obtain the blood sample and send it to the NIH. If it is not possible for any reason to ship the patient's blood to NIH, blood safety studies, specifically the CBC, differential, platelets, and CHEM 20 may be performed and the official report obtained from a certified clinical laboratory convenient to the patient's home. A note will be made on the History and Physical (H & P) DCF indicating the reason for the missed visit or alternative source of laboratory information.

Specimen collection kits will be mailed to the subjects if clinical evaluations are indicated and to be conducted at +/- 6 weeks of months 9, 15, 21, 27, 33, and 39. The subject and primary medical professional provider will be responsible for the collection and shipment of the blood specimen to the PI. A written instruction sheet for the collection and shipment of biological products will accompany these kits. As noted above, the PI or other AI will review this procedure with the subject prior to discharge.

Trial-related documents will be maintained for a period of 2 years after final marketing approval of the gene therapy product, or 2 years after the formal discontinuation of clinical development of the product per 21 CFR 312.57 and 21 CFR 312.62. The Principal Investigator must be aware of all requirements and retain protocol records in accordance with the longest requirement that pertains to the study. No study document should be destroyed without prior written agreement between the Principal Investigator and the NIAID. Storage of all trial-related documents will be such that confidentiality will be strictly maintained.

12.0 PROTOCOL MONITORING PLAN

The Site Monitoring Plan will be maintained in accordance to the specifications outlined by the monitoring organization. Monitors under contract to the NIAID will visit the clinical research site to monitor all aspects of the study in accordance with the appropriate regulations. During the course of the study, the independent study monitor will schedule site visits in order to monitor the quality and accuracy of collected data, to ensure the investigator is carrying out the agreed upon activities as stated in the approved protocol and to inspect the clinical site regulatory files to determine that all regulatory requirements surrounding the clinical are met. The usual frequency of site monitoring will be at least 3 times annually until the end of the study. The first site visit will occur within one month of the subject's enrollment. The site monitor will schedule a date to conduct an interim-monitoring visit that is mutually convenient for the PI and regulatory site staff. A letter of confirmation will be mailed to the PI and study coordinator stating the purpose and objectives of the visit, the staff and documents to be available for the visit, and the expected duration of the visit. The site will be responsible for retrieving any source documents required for the site visit. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit. Source documentation for all protocol-required information must be provided at the time the CRFs are reviewed. A review of the findings from the site visit will occur with the site staff upon completion of the visit. A site visit report will be sent to the PI/sponsor of the IND, study coordinator and Clinical Director, NIAID, within 2 weeks of the study visit. The site monitor will perform the following activities during an interim, monitoring visit:

a. Review regulatory documents

b. Review Drug Accountability

c. Review study data/records for accuracy and completeness.

d. Assure protocol is being conducted as specified.

e. Review Safety Reports including any adverse events.

The investigator (and/or designee) will make study documents (e.g., consent forms, data table pulls) and pertinent hospital or clinical records readily available for inspection by the local IRB, the FDA, the site monitors, and the NIAID staff for confirmation of the study data.

13.0 HAZARDS AND DISCOMFORTS

13.1 VENIPUNCTURE

Hazards associated with venipuncture include potential for pain, potential for hematoma formation and a small potential for infection at the site of needle stick. Occasionally, individuals undergoing venipuncture may faint as a result of a vasovagal reaction to the procedure. These risks will be minimized by carefully preparing the site of needle puncture with alcohol swab, by using the smallest size needle required for the procedure, and by having the patient lie down or sit as appropriate during and for 5 to 10 minutes after the venipuncture. The major risk of repeated blood drawing is the development of anemia from removal of too many red blood cells. To minimize this risk, the amount of blood drawn for all purposes will be adjusted so as not to exceed NIH Guidelines of 450 ml or 7.0 ml per kg body weight (whichever is less) over any six

week period to include blood draws for all purposes. EMLA or another topical anesthetic cream will be offered to the patient to minimize the discomfort of needle sticks.

13.2 CENTRAL LINE PLACEMENT

In the setting where the patient does not have a central line, one may be inserted upon admission. The rationale for the central line provides for the safe administration of busulfan, efficient central venous access for frequent phlebotomy requirements, while minimizing pain, discomfort, and the risk of infection to the patient from multiple catheter insertions to the veins of the arm. The risks from the central line placement procedure are low and include bleeding, bruising, or infection at the site of insertion. The risk of infection will be minimized by the use of proper sterile technique during the central line insertion procedure. Very rarely, pneumothorax may occur and/ or injury to an artery or nerve. Risks will be minimized by using only trained, experienced staff for the procedure and using local anesthetic or conscious sedation by either an anesthesiologist or other professional certified to administer conscious sedation.

13.3 BUSULFAN

Commonly listed side effects include nausea and vomiting, rash, pruritus, hyperglycemia, hypomagnesemia, seizure, hypophosphatemia, myelosuppression, mucositis, alopecia, infertility, veno-occlusive disease, teratogenesis, and neoplasia. The risk of seizure will be minimized by the administration of prophylactic anticonvulsant medication 30 minutes prior to busulfan treatment.

13.4 TRANSDUCED STEM CELL INFUSION: ALLERGIC AND VASCULAR REACTIONS, ANTIBODY FORMATION, AND INFECTION

Based on our experiences with a similar gene therapy protocol ((Dror, Gallagher et al. 1993; Messner 1998) and NIH protocol #95-I-0134) and procedure, no toxicities are expected from administration of the transduced stem cell product. It is possible for the patient to develop antibodies against any of the materials (including recombinant growth factors) used in the manufacture of the transduced stem cell product. The transduced CD34+ cells will be extensively washed and determined to be free of growth medium before being infused into the patient to further reduce the risk of reactions to components in the culture medium. Our SOP for culture and transduction of the cell product avoids the use of animal proteins and animal serum from our culture medium and retrovirus supernatants, which is an important safety feature that reduces the potential for allergic reactions (Malech, Horwitz et al. 1998; Malech 2000; Horwitz, Barrett et al. 2001). However, infusion of any type of blood cell product, even autologous cells, can be associated with reactions resulting from clumping (agglutination) of these cells or other immediate reactions related to sticking of these cells to blood vessels in the lungs. Potential reactions include shortness of breath, wheezing, low blood pressure, dizziness, and fever. The reactions are treated by stopping the infusion, and by providing oxygen, antihistamines, steroids, and medications or fluids to increase blood pressure. Because the infused cells in this study are autologous CD34+ cells and are still primitive progenitors at day 3 or 4, the potential for occurrence of agglutination or vascular reaction is very low.

It is theoretically possible for the CD34+ cell culture to become contaminated with microorganisms. One of the important safety features of this gene therapy approach is that the CD34+ HSCs will be grown and handled in sealed gas permeable flexible plastic containers (Malech, Bauer et al. 1997; Malech, Maples et al. 1997; Malech 2000). The closed bag system interfaces with a type of automated heat seal tubing connector that is the standard of practice in blood banks, thus reducing the possibility of microbial contamination. Microbiology cultures and/or gram stains will be performed on the cell medium two days prior to infusion, one day prior to infusion, and on the day of infusion. Endotoxin testing on the day of infusion must also be negative for the final cell product. However, the cells must be infused into the patient at the end of the 4^{th} day in culture (3 and 1/2 days from the start of the first transduction), before all culture results are final. Thus, the infusion of contaminated cells remains a remote possibility. Contaminated progenitor cell infusion would cause chills, fever, low blood pressure, and shortness of breath. The degree of this reaction or the subsequent spread of infection could even cause death. If the patient had a reaction to the infused cells that did not appear to have any contamination, we would stop the infusion and treat as medically indicated. Furthermore, if cultures later indicate such contamination, we would give the patient appropriate antibiotics and any other medically indicated treatment necessary to treat or prevent the infection as a result of this complication. An action plan to alert investigators of any microbial contamination of the transduced cell product is also available. Additionally, patients will be on antimicrobial treatment and will be maintained on all antibiotics during the treatment, further lessening the risk of a serious infection developing from the infusion of contaminated products. (For further details regarding sterility testing, Endotoxin assays, and management of contaminated products, please reference the Department of Transfusion Medicine, CC, NIH master file with the FDA, DMF Type V (BB-MF #11054), "Facility, Operational and Quality Systems for the Manufacture of Cellular Therapy Products, DTM, Magnuson Clinical Center, National Institutes of Health."

13.5 Quality Control and Risk Reduction With Transduced Cell Product Manufacturing

In order to ensure that the highest quality and safest transduced cell product is provided in this protocol, all culture, transduction and preparation (manufacturing) of all transduced cell products will take place in the specially designed and designated Cell Processing Laboratory within the Department of Transfusion Medicine (DTM) of the NIH Clinical Center under the direction of DTM staff with extensive knowledge and training in the preparation of such gene altered cell products.

The DTM Standard Operating Procedure for cell tracking and labelling, and the DTM Standard Operating Procedures for manufacturing of transduced hematopoietic stem cell product can be referenced to the NIH master file with the FDA, DMF Type V (BB-MF #11054), "Facility, Operational and Quality Systems for the Manufacture of Cellular Therapy Products, DTM, Magnuson Clinical Center, National Institutes of Health." In addition, the PI will communicate frequently with the DTM staff, directly observe portions of the manufacturing process, and inspect the procedures and records of the process to verify that the established manufacturing SOP is being followed. DTM will be ultimately responsible for release of the final cellular

product as per established DTM and NIH Clinical Center standards and procedures for release of blood products for administration to patients.

To ensure and document the quality and safety of the recombinant virus that will be used for the transduction portion of the manufacture of the transduced autologous CD34+ HSC, an extensive testing program has been established for the Master Cell Bank of the vector producer packaging clone 293-SPA-MFGS-gp91-155 packaging cells, and the production lot of the amphotropic envelope pseudo-typed MFGS-gp91 vector. These tests have been conducted in conjunction with a rigorous quality assurance program at BioReliance Corporation (14920 Broschart Road, Rockville, MD) who monitored all procedures and reagents used in the generation of these materials. All studies are negative (see Attachment 1, 1a, and 1b, pages 50-58) and the FDA lists the BB-IND-6100 holder as Dr. Malech.

14.0 BENEFITS, COMPENSATION, AND ALTERNATIVE THERAPIES

The patient, by participating in this protocol, may benefit from the production of normally functioning neutrophils. The larger the number of cells that are transduced and the longer the corrected cells remain detectable, the greater the benefit. The nature of the benefit includes increased ability to clear the current infection and prevent future infections. Since we do not know ahead of time how long the corrected cells will be detectable, it is not possible to determine the degree of benefit or time frame that this benefit will accrue. However, findings from Fischer's group showed that in 4 of 5 SCID patients treated with gene transfer the number of functional T cells noted *in vivo* were markedly increased after two years. *In vivo* antibody responses were also noted one year post-transfusion in these patients (Cavazzana-Calvo, Hacein-Bey et al. 2000; Hacein-Bey-Abina, Le Deist et al. 2002). We are hoping that improvements in number and quality of harvested CD34+ stem cells, along with the use of a high titre vector, and modifications in the preparation of the vector, as well as busulfan conditioning will result in at least partial correction of neutrophils, sufficient for cure of each subject's ongoing infection. If successful, gene therapy may also greatly improve the quality of the subject's life by decreasing the reliance on antibiotics and reducing recurrent hospitalizations.

Furthermore, a more general benefit to each patient's participation in this protocol will be the development of a better understanding of the utility of this gene therapy approach in the potential treatment of CGD.

15.0 REMUNERATION

The patient will not receive any monetary compensation as a result of participating in this trial. NIH will make arrangements for transportation to and from NIH along with provision for food and lodging for the patient and one parent while under treatment or evaluation at the NIH under this protocol. Provision of these arrangements will decided upon on an individual basis based on need and distance from the Clinical Center.

16.0 ALTERNATIVE THERAPIES

The only alternate therapy for CGD that, like gene therapy, has the potential to cure or substantially correct neutrophil oxidase activity is an HLA-matched sibling donor or HLA-matched unrelated donor (MUD) allogeneic transplantation. It appears that the replacement of the patients' hematopoietic system reduces or eliminates CGD related infections and improves or prevents CGD-related inflammatory events. However, there are no long-term data proving these treatments to be cures. More importantly, the patients in this study do not have an HLA-matched sibling donor. The risk of significant morbidity and mortality are very high for an unrelated transplant. Gene therapy would be considered of lower risk with equal possible benefit, and would not preclude the use of a MUD transplant should the gene therapy prove unsuccessful. The standard management utilizing long-term prophylactic antibiotics, which have been shown to reduce the incidence of bacterial infections in CGD patients, has failed in these patients and they are not surgical candidates for the treatment of their infectious processes. Beyond the aforementioned therapies, there are no other approved standard therapies for CGD.

17.0 PLAN FOR USE AND STORAGE OF BIOLOGICAL SAMPLES

This protocol requires the collection and storage of blood samples. Samples collected for clinical and research use will be stored in accordance with Good Clinical Practice. Samples will be issued a designated identifiable code number with limited access granted to authorized personnel to assure maintenance of patient confidentiality.

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19.0 PHARMACEUTICAL, BIOLOGIC, AND DEVICE INFO: BUSULFAN

Description

Source: ESP Pharma

Generic: busulfan

Other: Busulfex, Myleran (p.o. formulation)

Classification: Alkylating agent, Action Prevents cell division by altering DNA

Metabolism: Via the liver; sulfoxane, 3-hydroxysulfoxane, and other metabolites are formed and excreted in the urine.

19.1 TOXICITY

Hematological Effects: The most frequent serious consequence of treatment with busulfan at the recommended dose and schedule is profound myelosuppression occurring in all patients. Severe granulocytopenia, thrombocytopenia, anemia, or any combination thereof may develop. Frequent complete blood counts, including white blood cell differentials, and quantitative platelet counts should be monitored during treatment and until recovery is achieved. Absolute neutrophil counts dropped below $0.5x10^9$/L at a median of 4 days post transplant in 100% of patients treated in the busulfan clinical trial. The absolute neutrophil count recovered at a median of 13 days following allogeneic transplantation when prophylactic G-CSF was used in the majority of patients. Thrombocytopenia (<25,000/mm^3 or requiring platelet transfusion) occurred at a median of 5-6 days in 98% of patients. Anemia (hemoglobin <8.0g/dL) occurred in 69% of patients. Antibiotic therapy and platelet and red blood cell support should be used when medically indicated.

Pulmonary: Bronchopulmonary dysplasia with pulmonary fibrosis is a rare but serious complication following chronic busulfan therapy. The average onset of symptoms is 4 years after therapy (range 4-10 years).

Cardiac: Cardiac tamponade has been reported in pediatric patients with thalassemia (8/400 or 2% in one series) who received high doses of oral busulfan and cyclophosphamide as the preparatory regimen for hematopoietic progenitor cell transplantation. Six of the eight children died and two were saved by rapid pericardiocentesis. Abdominal pain and vomiting preceded the tamponade in most patients. No patients treated in the busulfan injection clinical trials experienced cardiac tamponade.

Neurological: Seizures have been reported in patients receiving high dose oral busulfan at doses producing plasma drug levels similar to those achieved following the recommended dosage of busulfan. Despite prophylactic therapy with phenytoin, one seizure was reported during an autologous transplantation clinical trial of Busulfex. This episode occurred during the cyclophosphamide portion of the conditioning regimen, 36 hours after the last busulfan dose. Anti-convulsant prophylactic therapy should be initiated prior to busulfan treatment.

Hepatic Effects: Current literature suggests that high busulfan area under the plasma concentration versus time curve (AUC) values (>1500uM*min) may be associated with an increased risk of developing hepatic veno-occlusive disease (HVOD). Patients who have received prior radiation therapy, greater than or equal to three cycles of chemotherapy, or a prior progenitor cell transplant may be at an increased risk of developing HVOD with the recommended busulfan dose and regimen. Based on clinical examination and laboratory findings, hepatic veno-occlusive disease was diagnosed in 8% (5/61) of patients treated with busulfan in the setting of allogeneic transplantation, was fatal in 2/5 cases (40%), and yielded an overall mortality from HVOD in the entire study population of 2/61 (3%). Three of the five patients diagnosed with HVOD were retrospectively found to meet the Jones' criteria. The incidence of HVOD reported in the literature from the randomized, controlled trials were 7.7% - 12%.

Others: Other reported adverse reactions include: headache (mild or moderate 64%, severe 5%), abdominal pain (mild or moderate 69%, severe 3%) asthenia (mild or moderate 49%, severe 2%), allergic reaction (mild or moderate 24%, severe 2%), injection site inflammation (mild or moderate 25%), injection site pain (mild or moderate 15%), chest pain (mild or moderate 26%), back pain (mild or moderate 23%), myalgia (mild or moderate 16%), arthralgia (mild or moderate 13%), and ear disorder in 3%.

Over dosage: There is no known antidote to busulfan. The principal toxic effects are bone marrow depression and pancytopenia but the central nervous system, liver, lungs, and gastrointestinal tract may be affected. The hematologic status should be closely monitored and vigorous supportive measures instituted, if necessary. Dialysis may be considered in the management of overdose, as there is 1 report of successful dialysis of busulfan. Busulfan is metabolized by conjugation with glutathione; thus, administration of glutathione may be considered.

Formulation and Preparation

Tetramethylene di(methanesulphonate); Butane-1,4-diol di(methanesulphonate).; supplied as an ampoule containing 60 mg/10 ml injection.

Stability and Solubility

Busulfan for injection should be refrigerated at 2 to 8 degrees Celsius.

Incompatibilities and Drug Interactions

Itraconazole decreases busulfan clearance. Phenytoin increases busulfan clearance by 15%.

Administration Procedure

IV busulfan must be diluted prior to use with either 0.9% Sodium Chloride Injection, USP (normal saline) or 5% Dextrose Injection, USP (D5W). The diluent quantity should be 10 times the volume of busulfan injection, so that the final concentration is approximately 0.5 mg/mL.

Busulfan will be administered intravenously via a central venous catheter as a 2-hour infusion on two consecutive days for a total dose of 10mg/kg. Antiemetics of the 5-HT3 class should be administered prior to the first dose of busulfan and continued on a fixed schedule through busulfan administration.

Phenytoin IV will be given daily for the two days of the infusion as anti-epileptic prophylaxis.

Biohazard Containment

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the Centers for Disease Control.

All infectious specimens will be sent using the ISS-1 SAF-T-PAK mandated by the International Air Transport
Association Dangerous Goods Regulations-Packing Instruction 602.
Please refer to individual carrier guidelines, e.g., Fed Ex, Airborne, for specific instructions.

APPENDIX A

TOXICITY TABLE FOR GRADING SEVERITY OF ADVERSE EVENTS (AGES 2-YOUNG ADULTHOOD)*

PARAMETER	GRADE 1	GRADE 2	GRADE 3	GRADE 4
7.2.1.1.0.1 HEMATOLOGY				
Hemoglobin (g/dl)	<LLN-10.0 g/dL	10.0-8.0 g/dL	<8.0-6.5 g/dL	6.5 g/dL
Abs Neutrophil Ct (k/mm3)	<1500/mm3	<1500-1000/mm3	<1000-500/mm3	<500/mm3
Platelets (thousand/mm^3)	75,000	<75,000-50,000	<50,000-25,000	<25,000
PT (seconds)	1.5-x1.5ULN	>1.5-2xULN	>2xULN	>2xULN
PTT (seconds)	1.5-2xULN	>1.5-2xULN	>2xULN	>2xULN
7.2.1.1.0.2 GASTROINTESTINAL				
Bilirubin (mg/dL)	>ULN-1.5xULN	1.5-3xULN	>3.0-10.0 xULN	>10.0xULN
AST (SGOT) (units/l)	>ULN-2.5xULN	>2.5-5.0xULN	.>5.0-20.0xULN	>20.0xULN
ALT (SGPT) (units/l)	>ULN-2.5 x ULN	>2.5-5.0xULN	.>5.0-20.0xULN	>20.0xULN
7.2.1.1.0.3 RENAL AND ELECTROLYTES				
Serum Creatinine (mg/dl)	>ULN-1.5xULN	>1.5-3.0xULN	>3.0-6.0xULN	>6.0xULN
High Serum Sodium (mmol/l)	>ULN-150 mmol/L	>150-155mmol/L	>155-160mmol/L	>160mmol/L
Low Serum Sodium (mmol/l)	<LLN-130mmol/L		<130-120mmol/L	<120mmol/L
High Potassium (mmol/l)			6.5-7.0	>7.0 or Cardiac arrhythmias

*Table adapted from the NCI CTC for Adverse Events (CTCAE) version 3.0, NIH and modified for CGD Patients and this protocol. ULN = upper limit of normal. LLN=lower limit of normal.

PARAMETER	GRADE 1	GRADE 2	GRADE 3	GRADE 4
Low Potassium (mg/dL)	<LLN-3.0 mg/dL		<3.0-2.5mg/dL	<2.5mg/dL
High Calcium (mmol/l)	>ULN-2.9 mmol/L	>2.9-3.1mmol/L	3.1-3.4mmol/L	>3.4mmol/L
Low Calcium (mmol/l)	<LLN-2.0mmol/L	<2.0-1.75mmol/L	1.75-1.50mmol/L	<1.50mmol/L
Low Magnesium (mmol/l)	<LLN-0.5mmol/L	<0.5mmol/L	0.4-0.3mmol/L	<0.3 mmol/L
Proteinuria (urinalysis)	Trace -1+ <0.15-1.0 g/day	2+ to +3 >1.0-3.5g/day	4+ > 3.5g/day	Nephrotic syndrome
Hematuria (urinalysis)	Microscopic <25 cells/hpf	Gross bleeding, urinary tract irrigation indicated	Transfusion, or other invasive intervention indicated to attain homeostasis	Life-threatening- urgent intervention indicated
Comments Calcium values are corrected for albumin concentration.				
OTHER (change at time of infusion)				
Allergy	Transient flushing or rash mild, localized	Intense pruritic Rash, flushing, may be localized or widespread	Intense Urticaria widespread, interfering with ADLs	Severe Urticaria Anaphylaxis, Angioedema
Fever (^{0}C)	< 38 C	>38.5-40 C	>40 C	Sustained Fever: >40 C
Cutaneous		Diffuse maculo-Papular rash, dry desquamation	Vesiculation, ulcers	Exfoliative dermatitis, Stevens-Johnson or Erythema multiforme, Moist desquamation, skin necrosis

OTHER (change during period of study follow-up)				
Allergy			Severe (>1 week) urticaria duration-unrelated to IVIG or other medications	Anaphylaxis, Angioedema
Wt. Loss	Loss of 3-5% compared to baseline	Loss of 5-10% compared to baseline	Loss of 10-20% compared to baseline	Loss of >20% compared to baseline
7.2.1.1.0.4 CENTRAL NERVOUS SYSTEM				
*New Onset Seizures	-	1 brief generalized uncomplicated Sz	seizures in which consciousness is altered; poorly controlled seizure disorder with "breakthrough" despite medical intervention	seizures of any kind which are prolonged, repetitive or difficult to control (status epilepticus)
Mental Status And Behavior	Changes which do not Affect Function	Changes requiring pharmacologic or other therapy; or mild lethargy, sedation or somnolence which resolves with rest	Changes not improved by drugs or other therapies; or onset of confusion, memory impairment, lethargy, sedation, or somnolence which does not respond to rest	Onset of delirium, obtundation, coma, or psychosis
Behavior refers to the development of attention deficits with or without hyperactivity, depression, mania, agitation, sleep disorders, phobias, obsessive-compulsive behaviors, or anxiety. Mental status refers to the level of consciousness, memory function, language and analytical operations, and non-dominant hemisphere functioning. Alternative explanations should be sought.				

APPENDIX B: Schedule of Evaluations: Patient Evaluations at Study Entry and During Period of Study

Evaluation / Parameter	Pre-Infusion *(–2 months to Month 0)*	Month 0 Day 0	M 1	M 2	M 3	M 6	M 9	M 12	M 15	M 18	M 21	M 24	M 27	M 30	M 33	M 36	M 39	M 42	M 48 (M X)
	Visit(s) *	V 1	V 2	V 3	V 4	V 5	V 6	V 7	V 8	V 9	V 10	V 11	V 12	V 13	V 14	V 15	V 16	V 17	V 18[a] (V X)
Informed Consent/Assent	X																		
Quality of Life Questionnaire	X				X	X		X		X		X		X		X		X	
Complete H&P, Wgt, Hgt, VS	X		X	X	X	X		X		X		X		X		X		X	X
EKG, Pulse Ox, CXR (or CT); CVC insertion	X																		
CHEM 20 Panel (1.5/4mL SST)	X	X[1]	X	X	X	X		X				X				X		X	X
CBC, diff., sed. rate (2.0/5mL)	X	X[1]	X	X	X	X		X		X		X		X		X		X	X
PT, PTT (2.7mL Blue)	X																		
Urinalysis	X					X		X		X		X		X		X		X	X
Screening serology (17mL) (HIV, Hepatitis, HTLV, TTV)[3]	X[4]																		
Transduced Autologous HSC Infusion		X[2]																	
Weekly phone contact[5]			X	X	X														
Interval History by phone[6]							X		X		X		X		X		X		
Busulfan Infusion[7]	X																		
Busulfan Levels[8] (16 mL total)	X																		
Serum sample stored (5 ml RTT)	X							X				X				X			X[b]
Blood Specimen for RCR[c] (2 GTT)	X				X	X		X				X[c]				X[c]		X[c]	X[c]
Cell surface markers (T, B, NK)[9] (4/5mL EDTA)	X				X	X	X	X		X		X		X		X		X	X
Qlg's = IgA, IgE. IgM and IgG(2mL/10mL SST)	X					X		X		X		X				X			X[b]
Cell-lineage separation[10] (10 mL GTT)	X				X	X		X				X[d]				X[d]			X[d]
Clonality of insertion sites if pro-virus detected, assay (LAM-PCR)					X	X		X				X[d]				X[d]			X[d]
DHR Assay (3 mL EDTA) (gp91 functional assay)	X				X	X	X	X[b]				X				X			X

H = medical history; P = physical examination; Wgt = weight; Hgt = height; VS = vital signs;

*: visits V 00, V 01, V 02, V 03, *etc.*, as required

[a]: visits (V X) being scheduled every 6-12 months (M X) after Month 48 (Visit 18) for 15 years.

[b]: and annually thereafter

[3]: including HBsAg (HBe if HBsAg positive), anti-HBc, anti-HCV and TTV (transfusion-trans-missible viruses) screen, including anti HTLV-1/–2, anti-HIV-1/-2 and HIVp24Ag.

[4]: if not tested within 2 months of HSC infusion

[5]: telephone interviews each week for the first 3 months (up to V 4) beginning on day of hospital discharge,

Evaluation / Parameter	Pre-Infusion *(–2 months to Month 0)*	Month 0 Day 0	M 1	M 2	M 3	M 6	M 9	M 12	M 15	M 18	M 21	M 24	M 27	M 30	M 33	M 36	M 39	M 42	M 48 (M X)
	Visit(s) *	V 1	V 2	V 3	V 4	V 5	V 6	V 7	V 8	V 9	V 10	V 11	V 12	V 13	V 14	V 15	V 16	V 17	V 18[a] (V X)

[c]: RCR=Replication Competent Retroviruses; after Month 12, if all RCR safety results are negative, annual sample stored; after Month 60, retrospective RCR testing if clin. indicated.

[d]: no test if no detectable provirus in any sample 3 and 6 months previously. If clone present, a 2nd test within 3 months, otherwise repeat annually for follow up

[1]: prior to hospital discharge

[2]: vital signs at –15, +15, +30, +60, +120min. pre/post infusion and prior to hospital discharge

ANC> 500, excluding weeks in which patient visits NIH for follow-up or is inpatient (total up to 12 except for visits on Month 1,2,3).

[6]: phone interview for months 9, 15, 21, 27, 33 and 39. If clinically indicated and/or requested by the PI, specimens may be collected via home blood collection kits

[7]: busulfan will be administered on Day –2 and Day –1

[8]: busulfan levels assessed on Day –2

[9]: flow cytometry: CD3, CD4, CD8, CD19 or 20, CD56, CD132, αβ TCR, γδ TCR-marking levels

[10]:to assay provirus insert by PCR in naïve vs. transduced of T, B, NK, monocytes, neutrophils

APPENDIX C: Gene Transfer for X-Linked CGD

SCHEDULE OF MONITORING FORMS

Form #	7.2.1.1.0.5 FORM NAME	Study Entry and Days –2/–1 Visits: V 00, V 01, V 02, V 03, etc.	Month 0 Day 0	M 1 V 2	M 2 V 3	M 3 V 4	M 6 V 5	M 9 V 6	M 12 V 7	M 15 V 8	M 18 V 9	M 21 V 10	M 24 V 11	M 27 V 12	M 30 V 13	M 33 V 14	M 36 V 15	M 39 V 16	M 42 V 17	M 48 (M X)* V 18 (V X)*
1	Eligibility Questionnaire	X	V 1																	
2	Laboratory	X		X	X	X	X	X	X		X		X		X		X		X	X
3	Infusion Record of Transduced-Cell Gene Therapy		X																	
4	Medical History and Medication Review by phone [1]	X						X[1]		X[1]		X[1]		X[1]		X[1]		X[1]		
5	Physical Examination, Medical History and Medication Review	X		X	X	X	X		X		X		X		X		X		X	X
6	Quality of Life Questionnaire	X				X	X		X		X		X		X		X		X	
7	Weekly Monitoring by phone (Medical History and Medication Review) [2]		X	X	X															
8	Missed Visit(s) [3]																			
9	Signature Form [4]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

*: visits (V X) being scheduled every 6-12 months (M X) after Month 48 (Visit 18) for the patient's life-time.

[1]: phone interview for months 9, 15, 21, 27, 33 and 39. If clinically indicated and/or requested by the PI, specimens may be collected via home blood collection kits

[2]: required weekly phone-contact post-infusion of gene therapy until V 4 (i.e., Visit 4 at Month 3)

[3]: as applicable

[4]: to be completed for each subject once all data collection forms are completed for each visit as part of Monitoring Plan.

Attachment 1
MFGS Vector and Production

Structure of Retrovirus Vector and Characteristics of Packaging Cells

For the generation of high titer recombinant viral vector encoding the $p47^{phox}$ cDNA or the $gp91^{phox}$ cDNA, we will use the MFGS retroviral vector and either the ψ-crip packaging cell line or the 293-SPA packaging cell lines. The MFGS-$p47^{phox}$ vector and ψ-crip packaging cell line combination were used to produce the vector supernatant used to treat the five $p47^{phox}$ deficient CGD patients in the first phase of the current protocol. This vector system (using MFGS-GM-CSF) was also used in clinical trials conducted by Cell Genesys for immunoadjuvant treatment of advanced renal cell carcinoma, prostate cancer and melanoma (FDA: BB IND 5229; RAC: 9303-040, 9408-082 and 9411-093). A large amount of cGMP grade clinical vector (MFGS-$p47^{phox}$ supernate) produced for the first phase of this trial is still in frozen storage and will be used for treatment of the patients with $p47^{phox}$ deficient CGD in this amended gene therapy protocol. These vector supernatant stocks will be tested to assure that the activity titers are still within the specifications before being used to treat additional patients. As noted above the $gp91^{phox}$ open reading frame cDNA has been cloned into the same MFGS vector backbone in exactly the same site and relationship as with $p47^{phox}$ and will be used for gene therapy of the X-linked form of CGD in this modified protocol. Much of the discussion below was originally composed to describe the MFGS-$p47^{phox}$ vector, but also relates exactly to the MFGS-$gp91^{phox}$ vector to be used in this modified protocol. The only difference is that the MFGS-$gp91^{phox}$ vector will be packaged in the newly engineered 293-SPA line (which is an human embryonic kidney cell line) rather than the ψ-crip line (which is derived from the mouse NIH 3T3 embryonic fibroblast cell line). 293 cells (which are the parent from which 293-SPA are derived) have been extensively used to produce the adenovirus vectors employed in a large number of clinical trials. The 293-SPA contains the same two CRIP packaging elements as the ψ-crip line. Therefore the discussion below about safety features of the packaging elements and vector backbone relate identically to both packaging lines when used to package either of the two phox subunit genes in the MFGS backbone.

MFGS Design and Structure of MFGS-$gp91^{phox}$:

MFGS is a derivative of the highly efficient MFG vector that incorporates additional safety features designed to minimize the potential for generating replication competent retrovirus. In the MFGS vector Moloney murine leukemia virus (Mo-MuLV) long terminal repeat (LTR) sequences are used to generate both a full length viral RNA necessary for the generation of viral particles and a splice modified subgenomic mRNA analogous to the Mo-MuLV *env* mRNA, which is responsible for the expression of the $gp91^{phox}$ genes. Unlike many vectors that were commonly in use, MFGS does not contain sequences encoding a selectable marker nor an internal promotor. The vector retains sequences in both the viral *gag* region shown to improve the encapsidation of viral RNA and the normal viral 5' and 3' splice sites necessary for generation of the subgenomic mRNA. It is of note that these two main features of MFGS (no selective marker from an internal promoter, and the retention of splicing elements to enhance protein production) are features that are being incorporated into a variety of other newly developed retrovirus vectors. Three point mutations have been introduced into the viral *gag* region to eliminate the potential expression of two overlapping open reading frames (ORFs), which encode the NH_2 portion of both the cell surface and cytoplasmic *gag-pol* polypeptides. These mutations effectively minimize the target region for recombination events that could lead to the generation of replication competent retrovirus, and theoretically minimize the potential for its formation.

Design of the 293-SPA packaging line:

The 293-SPA packaging cell line (for MFGS-$gp91^{phox}$ packaging), a derivative of the 293 adenovirus transformed human embryonic kidney cell line from the American Type Cell Culture repository, provides the viral proteins necessary for encapsidation of recombinant retroviral genomes into infectious particles. As is the case with other packaging cell lines, the expression of the relevant viral gene products in 293-SPA cells is accomplished in a manner designed to prevent the encapsidation and mobilization of the RNA molecules encoding the viral gene