いかぎり、環境中で増殖することはない。ヒト体内の同一の細胞にSF71gp91phoxとヘルパーウイルスが同時に感染する可能性は極めて低く、SF71gp91phoxが環境中に放出されたとしても、やがて消滅すると考えられる。

極めて微量のSF71gp91phox由来のRCRの環境中への放出も完全には否定できないが、レトロウイルス粒子へパッケージングできるRNAのサイズに上限があるため、RCRは野生型SFFVと同じになるか、あるいは短い外来遺伝子を含んでいても野生型SFFVに近い構造になると考えられる。RCRの感染性、増殖性、病原性及び核酸を水平伝達する性質は野生型ガンマレトロウイルスと同等であり、ヒトおよび他の哺乳動物、植物並びに微生物に新たな影響を与えることはないと考えられる。

従って、第一種使用規程承認申請書に記載した遺伝子組換え生物等の第一種使用等の方法によるかぎり、SF71gp91phoxによる生物多様性影響が生ずるおそれはないと判断される。

生物多様性影響評価書 別紙 目次

別紙１： SF71gp91phox の全塩基配列（下線部は gp91-*phox* コード領域）

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別紙２：ヒト gp91-*phox* のアミノ酸配列

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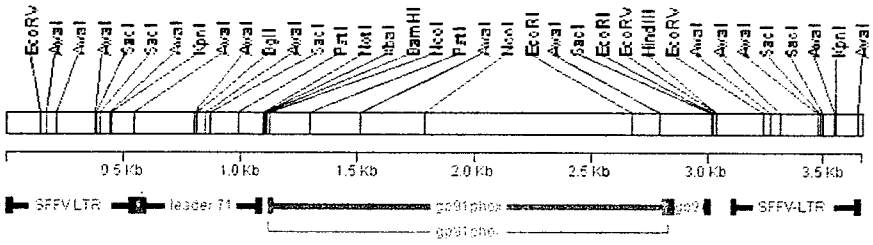
別紙３：ベクターの構造

SF71-gp91phox



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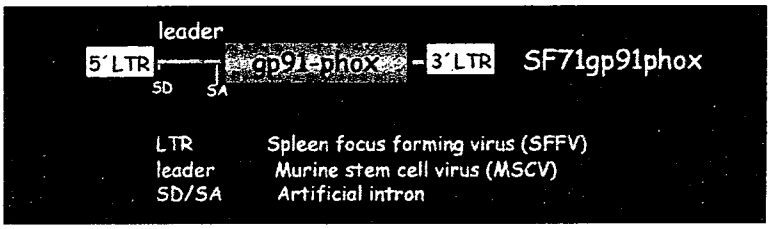
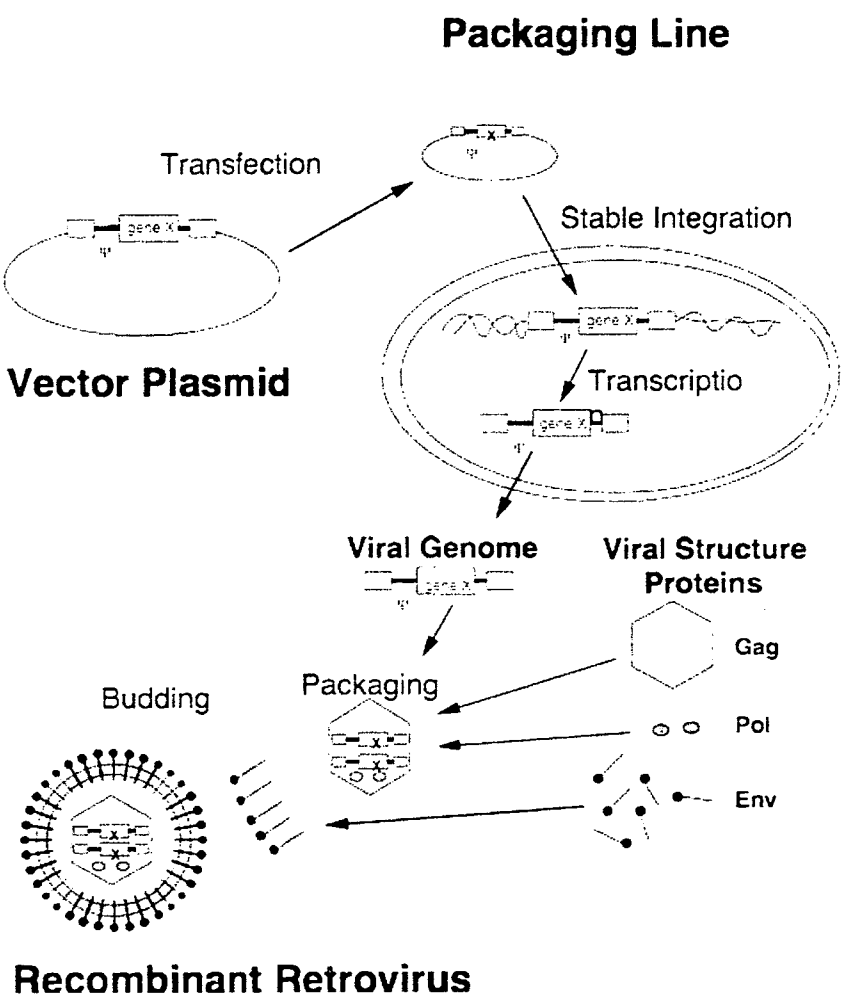


別紙５：組換えレトロウイルス作製の概略図



資料 3

AUTOLOGOUS TRANSPLANTATION OF GENETICALLY MODIFIED CELLS FOR THE TREATMENT OF X-LINKED CHRONIC GRANULOMATOUS DISEASE

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Medically Responsible Investigators:

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Version 1.0, dated 3/3/2006

NIH Protocol #:
FDA Drug/Device #: BB-IND 6100,
IND/IDE Holder (Sponsor): Harry L. Malech, MD

Key Words: Chronic Granulomatous Disease (CGD), CD 34 stem cells, busulfan, Gp91 phox deficiency, gene therapy, peripheral blood stem cells, immune reconstitution, bone marrow transplantation, engraftment.

Estimated Duration of Study: 1 year for enrollment, treatment and intensive assessment,; plus at least a 15-year long-term follow-up.

Subjects of Study	Number of Subjects	Gender	Age Range
Patients with Chronic Granulomatous Disease due to mutation of the GP91 phox gene	5	Male	3-55

Project involves ionizing radiation? No
Investigational Drugs? Yes, autologous stem cells transduced with an amphotropic pseudotyped MFGS gp91 expressing retroviral vector.
Off-Site Project? No
Multi-Institutional project? No

SCIENTIFIC PRECIS (Abstract)

This is a pilot protocol using moderate-dose busulfan as preconditioning prior to infusion of genetically modified cells using a retroviral vector (previously approved for use in a clinical trial under BB-IND 6100) for the treatment of X-linked Chronic Granulomatous Disease (X-CGD).

X-CGD is a rare, inherited immunodeficiency involving neutrophil function. X-CGD patients have neutrophils that lack production of oxidase, due to a mutation of the gene encoding for the gp91 phox. As a result, affected patients experience significant morbidity and a shortened life expectancy due to chronic infections and their associated treatments.

Currently, transplantation with either allogeneic or Matched Unrelated Donor (MUD) cells offer a potential cure. Gene therapy, unlike allogeneic or MUD transplantation, is not limited by donor availability or transplant complications, and is also potentially curative. To date, gene therapy has been used successfully in three different clinical trials to cure patients with immunodeficiencies related to a single genetic disorder including CGD. Eligible patients for this protocol are those without a matched sibling donor and who currently have an incurable infection using standard therapy. Without some form of treatment, using either a MUD transplant, or genetic correction of autologous stem cells, these patients will likely succumb to their ongoing infection. Therefore, as a possible treatment, we will use *ex vivo* transduction of the patient's own $CD34^+$ mobilized cells by culturing in cytokine supplemented media and MFGS-based retroviral vector supernatant. These cells will then be infused into the patient after conditioning with moderate dose busulfan

The primary clinical objective of this protocol is to offer gene therapy as a potential curative treatment to patients in urgent need of therapy, whose only alternative is a MUD transplant, due to the lack of eligible donor siblings. The primary scientific objective is to assess the safety and efficacy of a busulfan conditioning regimen in gene therapy. The endpoint criteria will be: the clinical resolution or stabilization of the patient's infection, no findings on all safety parameters; no reports of toxicity greater than grade 2 related to the infusion of busulfan conditioning; no reports of toxicity greater than grade 2 related to the cellular gene therapy product; the successful insertion of provirus into 5% or more of the patient's $CD34^+$ cells *ex vivo*; the detection of provirus in any mature blood lineage *in vivo*; the appearance of any neutrophils in the peripheral blood which express normal gp91 phox.

LAY LANGUAGE PRECIS (Abstract)

X-linked Chronic Granulomatous Disease (CGD) is an inherited disorder caused by an abnormal gene that fails to make the protein known as gp91 phox. This protein is part of a group of proteins that work to create hydrogen peroxide in neutrophils. Neutrophils are a type of white blood cell that helps fight infections. As a result, patients who do not make this gp91 phox frequently develop life-threatening infections. In addition, these neutrophils often act abnormally, resulting in the creation of a granuloma, which is an abnormal collection of cells. These granulomas can then become large enough to block organs, such as the bladder and/or intestines, causing significant problems. Patients are usually treated with antibiotics (often needed for extended periods of time) for the infections caused by CGD, and with corticosteroids for the granulomas. However, these drugs do not cure CGD itself, and can have significant side effects. Thus patients with CGD do not have a normal life expectancy.

The only available cure to date for CGD is Bone Marrow Transplantation (BMT), where the blood-making cells from a specially matched brother or sister donor (allogeneic) or a similarly matched unrelated donor are given to the patient after the patient has undergone some kind of chemotherapy or radiation in preparation for receiving the cells. If the cells from the donor engraft (or survive in the marrow), the patient can be cured; however, there is a risk that the cells may not engraft or that they may later get rejected from the body. Also, the cells from the donor can react against the patient, causing a serious disorder called "Graft Versus Host Disease" (GVHD). Although there are a number of methods used to try to reduce and/or prevent graft rejection and/or GVHD, these complications can still occur even with the newer methods now being developed. The risks of such complications are lower when a brother or sister is used as the donor; however, not all patients (even those with siblings) will have an ideally matched donor. Hence, transplantation, especially when using an unrelated donor, is not always a perfect cure.

Because the gene responsible for making the gp91 phox is known, it is possible to use gene therapy to try to cure this disease. In gene therapy, some of the blood-making cells are taken from the patient using a technique called apheresis. The normal gene is placed into the cells using special viruses called retroviruses. The cells are then able to produce the normal protein. In this trial, the patient will receive a small dose of chemotherapy called busulfan, lower than what is traditionally used in allogeneic BMT, and the newly corrected cells will then be put back into the patient.

Even with the best standard of care, a number of patients with CGD will still die from infection. For those patients who have an unresponsive or progressive infection and do not have a possible sibling donor, their only hope is either a Matched Unrelated Donor (MUD) transplant, which has a high risk of causing death itself, or gene therapy. Hence, we would propose using gene therapy in these patients as this has less risk of causing death, but can still possibly offer a cure. Even if the corrected cells do not remain lifelong to rid the patients entirely of their disease, as long as

they persist for even a few months, they would be able to at least clear the current infection for which the patients are being considered for enrollment in this protocol. Further, they would still be eligible to undergo a matched unrelated donor transplant in the event that gene therapy does not confer any benefit.

TABLE OF CONTENTS

ABBREVIATIONS LIST

Abbreviation	Text
Ag	antigen
AE	Adverse Event
AI	Associate Investigator
ANC	absolute neutrophil count
AST (SGOT)	aspartate transaminase
BMT	bone marrow transplantation
CBC	complete blood count
CGD	Chronic Granulomatous Disease
CNS	central nervous system
CRP	C-reactive protein
CT	computed tomography
CVC	central venous catheter
CXR	chest x-ray
DCF	data collection form
DHR	dihydrorhodamine assay
DLI	donor lymphocyte infusion
DSMB	Data and Safety Monitoring Board
DTM	Department of Transfusion Medicine
ESR	erythrocyte sedimentation rate
flt-3	fetal liver tyrosine kinase 3-ligand
GCP	Good Clinical Practice
GCSF	granulocyte colony stimulating factor
GVHD	graft versus host disease
H&P	history and physical
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HSC	hematopoietic stem cell
HVOD	hepatic veno-occlusive disease
IBC	Institutional Biosafety Committee
ICH	International Conference on Harmonisation
IgM, IgA, IgE, IgG	Immunoglobulins M, A, E, G
IL	interleukin
IRB	Institutional review board
LHD	Laboratory of Host Defenses
MDGF	megakaryocyte derived growth factor
MFGS	Mutated form of replication-incompetent retroviral vector derived from Moloney murine leukemia virus
MUD	matched unrelated donor

Abbreviation	Text
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
OBA	Office of Biotechnology Activities
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PI	Principal Investigator
PIN	patient identification number
PT	prothrombin time
PTT	partial thromboplastin time
RBC	red blood cells
RCR	replication competent retrovirus
RNA	ribonucleic acid
SAE	serious adverse event
SC	supportive care
SCF	stem cell factor
SCID	severe combined immunodeficiency
TPO	thrombopoietin
TTV	transfusion-transmissible virus
UA	urinalysis
WBC	white blood cell count
X-CGD	x-linked chronic granulomatous disease

1.0 INTRODUCTION

1.1 BACKGROUND

Chronic Granulomatous Disease (CGD) is one of a group of inherited disorders affecting neutrophil function. Due to a mutation in any of four genes encoding subunits of the phagocyte NADPH oxidase, patients with CGD are susceptible to recurrent bacterial and fungal infections. Besides life-threatening infections, these patients develop granulomas that can result in genitourinary or gastrointestinal tract obstruction, inflammatory bowel disease, retinal lesions, surgical wound dehiscence, and other manifestations of inflammation. Iatrogenic or infection-related end-organ damage also occurs in long-term survivors of CGD. Despite improvements in infection prevention and control, 2-5 percent of patients with the severe form of CGD die each year and few patients survive to age 50. (Winkelstein, Marino *et al.*, 2000).

The U.S. registry of CGD patients shows that about 75% of patients have the X-linked form of CGD, which involves mutations of the gp91 phox gene present on the X chromosome. About 20% of CGD patients have autosomal recessive CGD resulting from mutations in the p47 phox gene present on chromosome 7. Three percent and 2% of CGD patients have autosomal recessive disease resulting from mutations in either p67 phox (chromosome 1) or p22 phox (chromosome 16), respectively. CGD registry survival data indicates that X-linked CGD patients have a higher rate of infections and higher mortality (about a 3-5% death rate per year) than the p47 phox deficient autosomal recessive CGD patients (about a 1-2% death rate per year). The subnormal amount of oxidants produced by stimulated neutrophils in patients with CGD may vary from patient to patient. Neutrophils from patients with X-linked CGD who produce no detectable gp91 phox protein tend as a group to produce little or no detectable oxidants. By contrast, neutrophils from patients with X-linked CGD who make some detectable abnormal gp91 phox and patients with the p47phox deficient autosomal recessive form of CGD tend to produce modest amounts of oxidants. It is likely that the amount of trace oxidants produced by a CGD patient's neutrophils might affect susceptibility to infection.

Infections in CGD patients have a variety of presentations. Soft tissue infection or osteomyelitis with *Serratia* species is the most common presentation in infants (< 1 yr. old). Lung, lymph node, bone or skin infection with *Burkholderia* species, *Nocardia,* or *Aspergillus* is a common presentation leading to the diagnosis of CGD in older children or adults. Infections may be associated with fever, neutrophilia, high erythrocyte sedimentation rate (ESR) or high C-reactive protein (CRP), or malaise may be the only symptom. Fungal infection (50% of CGD infections) including pneumonia is indolent, often presenting with only malaise (or even as an incidental finding of a small lung infiltrate on routine radiographic study). After pneumonias, liver abscesses are the second most common severe deep tissue infection of CGD patients. In approximately 90% of liver abscesses *Staphylococcus aureus* is the causative agent.

In addition to infections, patients with CGD also suffer from problems related to granuloma formation. Approximately 50% of CGD patients have recurrent episodes of gastrointestinal granuloma formation (chronic abdominal discomfort, intermittent vomiting, and even frank obstruction). Twenty percent have inflammatory bowel disease (manifested by chronic diarrhea and/or rectal abscess, fissure or fistulas). In addition, approximately 10% of CGD patients have one or more episodes of acute bladder and/or ureteral obstruction. Wound dehiscence after surgery may be another manifestation of granuloma formation. Retinal granuloma lesions can also be observed in many patients, where the lesions are asymptomatic but may sometimes be

associated with scotoma on visual field analysis or be associated with abnormalities of measures of retinal neuro-electrical activity.

1.2 CURRENT TREATMENTS FOR X-LINKED CGD

1.2.1 Antimicrobial, Antifungal, and Other Prophylaxis Regimens

Long-term prophylactic antibiotic use has been demonstrated to reduce the incidence of bacterial infections. Liese *et al.*, (Liese, Kloos et al. 2000) reported a reduction of severe bacterial infections (*i.e.*, requiring hospitalization and IV antibiotics) from 4.8 to 1.6 per 100 patient months in subjects receiving trimethoprim-sulfamethoxazole (TMP-SMX, 5 mg/kg) for at least 12 months. Furthermore, in a subset of 8 subjects at high risk for *Aspergillus* infections being treated with 5.1 mg/kg of itraconazole, no fungal infections were observed. Mouy *et al.*, also noted a similar reduction in fungal infections (0.115 to 0.034 *Aspergillus* infections/year) in subjects being prophylactically treated with itraconazole (Mouy, Veber et al. 1994). In this study, due to insufficient plasma drug levels, the dose was increased from 5 to 10 mg/kg. Both studies reported no drug-related side effects except for transient elevations of liver enzymes that returned to normal without modification of prophylaxis treatment. A more recent study of itraconazole versus placebo similarly showed a reduction in serious fungal infections (Gallin, Alling et al. 2003). In this study one of the 39 subjects taking itraconazole acquired a serious fungal infection compared to seven of the 39 subjects on placebo during the 113 patient-years of follow up. The authors reported that three subjects were withdrawn from the study due to itraconazole-related adverse events, which included elevated liver enzymes, headache and rash.

Prophylactic antibiotic coverage with TMP-SMX and itraconazole are recommended for all patients with CGD followed at the NIH. Interferon gamma therapy has also been shown to decrease the incidence of infection and is, therefore, part of the recommended prophylactic regimen (NEJM, 1991). Based on several clinical studies, CGD patients in the United States are routinely placed on a prophylactic infection prevention regimen consisting of the following medications:

a. Daily oral trimethoprim-sulfamethoxazole (co-trimoxazole: about 4.6 mg/kg/day Trimethoprim - 22.8 mg/kg/day sulfamethoxazole for bacterial prophylaxis)
b. Daily oral itraconazole (about 4 mg/kg/day for fungal prophylaxis)
c. Three times weekly subcutaneous injections of recombinant interferon gamma (50 micrograms per meter squared surface area)

Patients who are allergic to either of the components of co-trimoxazole (usually the sulfa component) are placed on daily prophylaxis with any of a variety of other oral antibiotics, but most commonly either a fluoroquinolone (ciprofloxacin, levofloxacin or moxifloxacin) or a broad-spectrum cephalosporin (or rarely linezolid at 600 mg daily in an adult together with pyridoxine to prevent neurological side effects), with the primary aim being to prevent infection with *Burkholderia* species, *Serratia*, *Staphylococcus* and *Nocardia*.

For patients who are intolerant of itraconazole (primarily chemical hepatitis), there are no safe oral alternatives that provide prophylaxis against *Aspergillus* and other molds. For some patients who have had recurrent fungal infections despite itraconazole prophylaxis,

voriconazole used at 4mg/kg twice a day has been substituted for itraconazole as a routine daily prophylaxis.

1.2.2 Hematopoietic Stem Cell Therapies for CGD

There has been increasing use of bone marrow or peripheral blood stem cell transplantation (either a Human Leukocyte Antigen [HLA]-matched sibling or HLA-matched unrelated donor [MUD]) to treat CGD, using either non-myeloablative (Horwitz, Barrett *et al.*, 2001) or more conventional myeloablative approaches (Seger, Gungor *et al.*, 2002). This has resulted in a significant cohort of CGD patients (5 transplanted at NIH and currently followed at NIH; many others throughout the United States) who have had their neutrophil oxidase activity substantially corrected or even cured. While observation of these patients appears to confirm that replacement of their hematopoietic system reduces or eliminates their CGD-related infections and improves or prevents their CGD-related inflammatory events, statistical proof is lacking that transplantation actually achieves these goals.

1.3 RATIONALE

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 for this study have none. Although the use of MUD transplantation improves the chances of finding a suitable donor, the risks of graft rejection and/or graft-versus-host disease (GVHD) are increased, and both related and unrelated transplants continue to have significant risk. 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 future use of a MUD transplant.

1.4 RESULTS OF PREVIOUS TRIALS

Newer non-myeloablative regimens are being developed with the hopes of improving outcomes. Despite the fact that the incidence of acute GVHD has apparently decreased, chronic GVHD and graft rejection continue to limit the use of these sub-ablative regimens. This, too, applies to umbilical cord blood transplants, which have shown some early promise in reducing GVHD; however, these transplants are limited to patients of smaller size due to graft size considerations, and graft rejection remains a problem. Further complicating the applicability of cord blood is the current lack of a national cord blood collection or matching program. Hence, finding products is dependent on single institutions, and protocols remain experimental. Patients to be recruited to this study have no siblings and the risk of undergoing transplantation is magnified due to the underlying infection for which the patient is made eligible under this protocol.

1.4.1 Gene Therapy

Gene therapy has been often touted as a possible cure for genetic disorders. A number of small animal studies, including xenogeneic models, have shown proof of principal using vectors corrective for both the p47 mutation and the gp91 mutation (Mardiney, Jackson *et al.*, 1997; Malech, Horwitz *et al.*, 1998). Results of a Phase I clinical trial of gene therapy for patients with