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Clinical Protocol

Intracellular Antibodies Against HIV-1 Envelope Protein for AIDS Gene Therapy

A pilot study to evaluate in HIV-1 infected humans the safety and effects of autologous lymphocytes transduced with a human single-chain antibody against HIV-1 envelope protein

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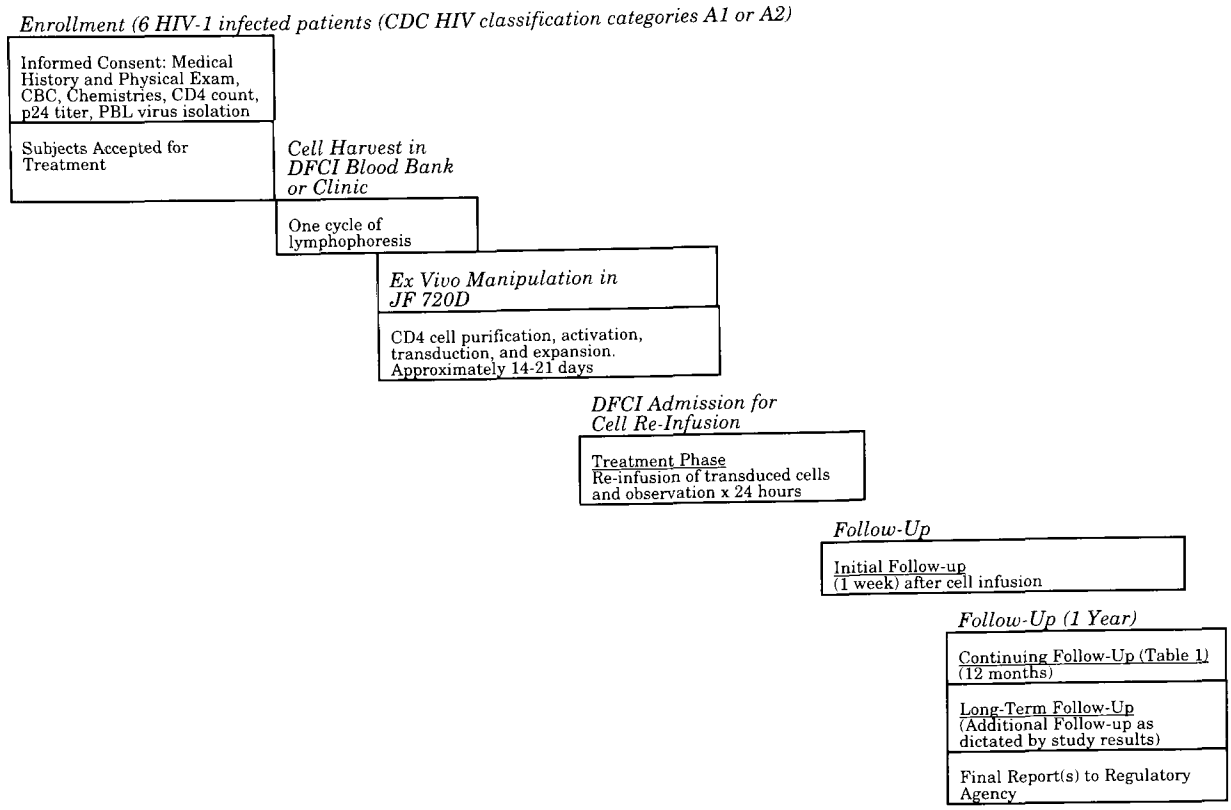

I. Introduction

Background and Rationale for Gene Therapy of HIV Infection

A. The Nature and Magnitude of the HIV-1 Pandemic

Human immunodeficiency virus (HIV) productively infects human $CD4^+$ cells, including $CD4^+$ T cells, cells of the monocyte/macrophage lineage, and other cell types, such as neuronal cells (1,2). Infection with HIV-1 leads, in most cases, to a progressive decline in the number and functions of $CD4^+$ T cells with the eventual appearance of clinical manifestations of cellular immunodeficiency, such as opportunistic infections and malignancies—i.e. AIDS (3–5). Over a decade after the first description of AIDS was reported, the global pandemic continues to grow. The World Health Organization has estimated that there are 12 to 14 million HIV-infected persons worldwide in 1993, with heavy concentrations in developing countries in Africa and Southeast Asia (6,7). Worldwide, it has been estimated that more than 1 million people have died of AIDS and over 1 million are living with symptomatic AIDS (7,8).

The Centers for Disease Control and Prevention (CDC) has estimated that there are approximately 1 million HIV-positive persons in the United States (7,8), most of whom are asymptomatic and many of whom are unaware of their seropositivity. In 1992, HIV infection became the number one case of death among men aged 25–44 years (9). While homosexual and bisexual men and intravenous drug abusers have accounted for most of the cases, the number of cases of AIDS resulting from heterosexual transmission of HIV has been steadily increasing (7). Current estimates indicate that women now represent 12% of AIDS cases and that 75% of these women are in the child bearing age (10). HIV-1 infection in women results in the birth of approximately 1800 infected infants in the U.S. annually (11). Furthermore, as recent data suggest that the proportion of infected women is increasing, the number of infected children is expected to increase accordingly. In 1994, AIDS in the United States is expected to increase to approximately 60,000–70,000 new cases per year (12).

B. The Natural History of HIV-1 Infection

Infection with HIV usually progresses from initial seroconversion to apparent clinical latency, followed by progressive immunodeficiency, opportunistic infections, malignancies, and death (13). Although an average period of 10 years exists from seroconversion to advanced symptomatic disease, the rate of progression varies widely with the "latent period" being less that 1 year in some persons or longer than 10 years in others; the reasons for such broad differences are not yet understood (7,13). The infection, although asymptomatic, is not truly latent because this period is characterized by a gradual reduction in CD4 cell counts (7). In addition, recent reports have provided strong evidence that in early-stages of HIV-1 disease there is a dichotomy between the levels of viral burden and virus replication in peripheral blood versus lymphoid organs. HIV disease is active in the lymphoid tissue throughout the period of clinical latency, even at times when minimal viral activity is demonstrated in blood (14,15). These results raise the possibility that early in the course of HIV-1 infection at a time when viral burden is low in the PBLs, lymphoid organs serve

as major reservoirs for HIV-1 and the spread of HIV-1 may occur predominantly in the microenvironment of the lymphoid organs.

C. Discussion of Animal Models of AIDS

Ideally, animal models of AIDS could serve to test the efficacy of strategies for eliciting protective immunity against HIV. However, a readily available laboratory animal that can be infected with HIV-1 and that will develop an AIDS-like illness does not exist despite an extraordinary effort to develop one (16). The only species that can be reliably infected with low doses of HIV-1 are the great apes. Yet, although chimpanzees and gibbons can be infected with HIV-1, they do not manifest an AIDS-like disease (16–18). In addition, they are an endangered specie and are expensive (16).

Another primate animal model that has been established to study AIDS and vaccine approaches is the infection of macaques with HIV-2 or various simian immunodeficiency viruses (SIV) (19–21). SIV infection of macaques displays striking similarities to the human HIV-1 infection with regard to disease development (22), whereas HIV-2-infected macaques remain symptomless (23–25). However, these animals do not develop immune responses analogous to humans infected with HIV-1 (26). In particular, the V3 loop of SIV envelope, in contrast to that of HIV-1, does not elicit neutralizing antibody (26,27).

The feline immunodeficiency virus (FIV)/CAT model has recently been evaluated for use in anti-HIV drug evaluation (28). Similar to HIV-1, FIV has an asymptomatic stage, a long latency period of approximately six years, depletion of $CD4^+$ cells, opportunistic infections, various malignancies, and wasting. However, because of the marked differences in the envelope sequences of FIV, an intracellularly expressed anti-FIV envelope antibody recognizing a totally different target sequence would have to be evaluated and would not be truly indicative of either efficacy or risk of administration of the intracellular anti-HIV-1 envelope antibody that we propose to use (see below, Section E).

Chimeric mouse models (SCID, *bg/nu/xid*, bone marrow-ablated BALB/c) reconstituted with human PBMCs, lymph nodes, or fetal liver/thymus tissues can be infected with HIV-1 but results in a disease that resembles HIV-1-infection rather than AIDS (28–30). Nevertheless, in studies that are planned, we will study the acute and subacute toxicities of the anti-HIV-1 envelope single chain antibody, termed sFv105, in an *in vivo* setting using the SCID-Hu model in collaboration with Dr. Robert Finberg (DFCI). Human PBLs will be transduced *ex vivo* as in our other studies and subsequently tested for resistance to viral challenge *in vivo* in the mice.

In summary, the lack of an animal model for AIDS, despite an intense search for one, has led to a broad consensus that many questions critical to experimental HIV-1 vaccine and treatment development must be answered directly in human trials (31).

D. Rationale for Gene Therapy of HIV-1 Infection

In 1993, a State-Of-The-Art conference, was convened by the National Institute of Allergy and Infectious Disease to evaluate the role of nucleoside analogue reverse transcriptase inhibitors (AZT, ddI, ddC) in the treatment of HIV-1 infection. The finding of the panel underscored the emerging concerns of the limited effectiveness and durability of response to these drugs (13), although, other recent advances have enabled physicians to prolong symptom-free intervals by providing improved treatments of opportunistic infections (7). At route of the limited effectiveness of the reverse transcriptase inhibitors may be the inherent difficulty of treating "retroviral infections." Following HIV-1 infection, reverse transcription of the viral RNA and permanent integration of the resulting DNA into the genome of the infected cell occurs (32). There the integrated provirus becomes an "inheritable" feature of the cell and retains the potential for viral expression and production of progeny virus for the duration of the cell's lifetime. In essence, HIV infection results in an acquired genetic disease which may be best treated by a genetic based strategy.

E. Rationale for Intracellular Antibody Gene Therapy and Previous Data with the Intracellular Antibody against HIV-1 Envelope Protein

Many of the pathogenic mechanisms associated with HIV-1 infection that lead to clinical disease have been established, such as the functional abnormalities and quantitative depletion of $CD4^+$ T lymphocytes that cause the profound immunosuppression characteristic of advanced HIV-1 infection (33,34). It is generally agreed that HIV-1 itself has the primary role in the initiation of the pathogenic process, however, a number of important issues concerning the pathogenesis of HIV-1 infection and disease from both a virologic and an immunologic standpoint remain unresolved and has stimulated investigation into additional mechanisms of immune dysfunction associated with HIV-1 infection (33,34).

In general, acute HIV-1 infection of cell cultures is characterized by typical cytopathic effects manifested by vacuolization of cells and formation of syncytia and consequently cell lysis (35). The envelope glycoproteins of HIV-1 have been implicated in the cytopathic effect of the virus, which is specific for cells bearing high levels of the virus receptor CD4 (36). In tissue culture, the cytopathic effects of HIV-1 consist of multinucleated giant cell (syncytium) formation and the lysis of single cells (37,38). Syncytium formation is mediated solely by the HIV-1 envelope expressed on the infected cell surface (39,40). The envelope binds at the CD4 receptor present on adjacent cells and then, via a fusion reaction analogous to that involved in virus entry, the opposed cell membranes are fused so that heterokaryons are formed.

Single cell lysis also depends upon efficient membrane fusion induced by the HIV-1 envelope glycoproteins, as some mutations in the gp41 amino terminus result in replication-competent viruses attenuated for both syncytium formation and single cell lysis (41). The observations that amino acid changes in gp120 that effect processing of the gp160 precursor can decrease single cell lysis (42) and that single cell lysis requires adequate levels of CD4 expression, independent of a level of viral protein expression or viral DNA in the infected cell (36), also implicate the HIV-1 envelope glycoproteins in this process. In the presence of HIV-1 gp160, a ternary complex of gp160-CD4 and $p56^{lck}$ (a tyrosine kinase that binds to the cytoplasmic tail of CD4) forms and is retained in the ER and this mislocation of tyrosine kinase to the cytoplasmic face of the ER may play a role in single cell lysis (43).

Various hypothesis other than direct HIV-1 infection have

been proposed to explain the profound immunodeficiency these patients can develop (33,34). For example, it has been demonstrated that a subset of $CD4^+$ gp120 specific clones manifest cytolytic activity and lyse uninfected autologous $CD4^+$ T cells in the presence of gp120 in a process that is strictly dependent on CD4-mediated uptake of gp120 by T cells (44). Assuming gp120 is shed from HIV-1-infected cells *in vivo*, this CD4-dependent autocytolytic mechanism may contribute to the profound depletion of $CD4^+$ T cells in AIDS. In a model system data has been presented that shows that an autoimmune idiotypic network develops in HIV-1 infection which leads to the development of autoimmune antibodies that destroy $CD4^+$ T cells (45,46). The autoimmune mechanism develops because of the sequence homologies between gp120 and class II MHC molecules (45–47). The immunosuppressant effects of gp120 on the $CD4^+$ T cell proliferation to antigenic stimulation has been demonstrated (43,48–50). These studies suggest that HIV-1 may affect major histocompatibility complex II restricted antigen recognition independent of $CD4^+$ T cell loss. In rodent neurons, gp120 has been shown to cause an increase in intracellular calcium and neuronal toxicity (51), an effect which might be mediated by activation of the nuclear endonuclease. In addition, activation-induced T cell death or apoptosis, has also been proposed to occur *in vivo* and accounts for the progressive depletion of $CD4^+$ T cells that leads to AIDS (52,53). *In vitro* and *in vivo*, soluble gp120 may interact with CD4 receptors on uninfected cells, leading to an abortive cell activation and thus trigger apoptosis (54–56). gp120 may also act as a superantigen, thereby leading to massive stimulation and expansion of T cells bearing specific beta regions followed by depletion or anergy (33). The superantigen hypothesis regarding HIV-1 infection stems from the observation that endogenous or exogenous retroviral-encoded superantigens stimulate murine $CD4^+$ T cells *in vivo*, leading to the anergy or depletion of a substantial percentage of $CD4^+$ T cells that have a specific variable beta region (57,58). In line with this hypothesis, there have been reports that patients with HIV-1 infections had perturbations in T cell subgroups bearing certain specific variable beta regions. More recently, gp120 has been implicated as a superantigen for B cells bearing VH3 heavy chain variable region genes (59). **Collectively, these data suggest that a decrease in the burden of gp160/gp120 in the HIV-1-infected patient may lead to improved immune cell and non-immune cell functions.**

Towards our long-term goal of delivering a therapeutic antiviral gene to HIV-1 infected or uninfected $CD4^+$ cells, we have recently reported on a new class of molecules that should be useful for AIDS gene therapy. Antibodies have the ability to recognize a very wide variety of molecules. Upon synthesis, antibodies are normally secreted into the extracellular fluid or remain membrane bound on the B cell surface as antigen receptors (60). Recent advances in antibody engineering have allowed antibody genes to be manipulated and antibody molecules to be reshaped (61). These advances raise the possibility that antibodies can be made to function intracellularly, to bind and to inactivate molecules within the cells. The feasibility of using intracellular antibodies, termed intrabodies, to inhibit the function of a cellularly expressed viral protein has been illustrated using as a target the envelope glycoprotein specified by HIV-1 (62,63).

The antibody used for these studies was the broadly neutralizing human monoclonal antibody, F105 that competes with CD4 for binding to gp120 (64,65). Mutagenesis studies indicate that the binding sites on gp120 for CD4 and F105 overlap (66). The F105 antibody binds to the envelope glycoprotein of multiple HIV-1 strains including many primary isolates (64,67). The strategy followed was to create a correctly folded molecule that contains both the heavy and light chain variable regions of the F105 antibody that are joined by an interchain linker and to direct this protein (termed sFv105) to the lumen of the endoplasmic reticulum. The sFv105 intrabody is designed to react to the nascent folded envelope protein within the ER and to prevent transit of the envelope antibody complex to the cell surface. In these studies, the intrabody was stably expressed and retained in the endoplasmic reticulum and was not toxic to the cells. sFv105 binds to the envelope protein with the ER and inhibits the cleavage and maturation of gp160. Cleavage of gp160 to gp120 is required to produced infectious HIV-1 virions (68). In addition, the sFv105 expressed in cells showed marked diminished syncytium formation that is associated with expression of this critical viral protein. Moreover, the infectivity of the HIV-1 particles produced by cells that expressed the sFv105 intrabody was substantially reduced. In quantitative assays, the viruses were 1,000 to 10,000-fold less infectious. In these experiments the sFv105 was driven by the CMV immediate early promoter (62).

More recently, an inducible expression vector was constructed in which the sFv105 intrabody is under the control of the HIV-1 long terminal repeat (LTR)/promoter (63). The sFv105 is inducibly expressed after HIV-1 infection or in the presence of HIV-1 *tat* protein and is retained intracellularly. A human $CD4^+$ lymphocyte cell line, SupT1, transformed with the expression vector exhibited resistance to virus-mediated syncytium formation and a decreased ability to support HIV-1 production. Surface gp120 expression was markedly reduced and surface CD4 was restored to normal following HIV-1 infection in the transformed lymphocytes. Cell surface phenotype, replication rate, morphology, and response to mitogenic stimulation of the transformed cells were also normal. The finding that the downregulation of the surface CD4 by HIV-1 was not observed in the lymphocytes intracellularly expressing sFv105, strongly suggests that the surface CD4-downregulation is mainly caused by intercellular CD4-gp160 complex formation which is likely blocked by the expressed sFv105 (63). The sFv105 intrabody, therefore, may not only inhibit virus production but also the virus cytopathic effects by blocking the interaction of envelope and CD4.

The F105 intrabodies are also able to inhibit infectious HIV-1 production by binding intracellularly to envelope mutant viruses that escape neutralization by extracellular antibody (69). These results suggest that the expressed intrabodies can intracellularly interfere with the function of envelope mutants that escape neutralization to extracellular F105 antibody because of the high concentration of expressed F105 intrabodies in the secretory vesicles. As a result, the intracellular blocking of envelope protein maturation may be accomplished by simply binding of the intrabody to envelop escape mutants.

In the several cell types that we have examined, the HIV-1 virions released from the intrabody expressing cells have been markedly diminished in their infectivity. When quantitatively

compared in the sensitive SupT1 syncytia forming assay, the virus particles have reduced infectivity by 10^3- to 10^4-fold. Thus, this unique form of gene therapy works by not only protecting the infected cells from cytopathic effects of gp120 but also produces virions that are markedly less infectious. The prepublications detailing these previous results are attached (Appendix E) (62,63,69).

A method that has been used successfully to generate high titer retroviruses has been an initial transfection of an ecotropic packaging cell line such as the line PE501 followed by collection of retrovirus-containing supernatant from these cells that is then used to infect the amphotropic cell line, PA317. Direct transfections of the ecotropic PE501 cell line using calcium phosphate coprecipitation are used to introduce the vector DNA. One to two days later the virus-containing medium are removed and centrifugated to remove cells and debris (2,000 rpm), followed by filtration through a 0.45 μm filter. The PA317 cells are then exposed to the virus-containing media for 24 hr in the presence of 4 μg/ml polybrene or protamine sulfate (SIGMA). The cells are grown in HAT selective median (Hypoxanthine 1×10^{-4} M, Aminopterin 4×10^{-7} M, Thymidine 1.5×10^{-5} M with 10% fetal calf serum in DMEM), which indirectly selects for expression of the packaging genes. Two days after infection, the PA317 cells are subjected to selection in 400 μg/ml G418, which selects for expression of the neomycin phosphotransferase gene that is expressed from the viral LTR promoter in the LN series of vectors. Individual clones are tested for production of high titer retroviruses by titering on NIH 3T3 cells, using the G418 selection to screen for effective gene transfer. The PA317 and PE501 are derived from NIH/3T3 thymidine kinase negative cells.

The PA317 and PE501 packaging cell lines were provided by Fred Hutchinson Cancer Research Center, Seattle WA. The cell lines have been extensively described in previous RAC approved gene therapy protocols. We have isolated clones producing retroviral titers between 10^5 PFU/ml and 10^6 PFU/ml as measured on NIH/3T3 cells.

Supernatants from subclones containing the replication-defective retroviruses were used to infect COS and SupT1 cells and were shown to express the sFv105 intrabody. Clones that produce the highest NeoR and sFv titer will be used as the vector producing cell line to be included for use in the clinical studies. The generation of retroviral vectors from transfected PA317 cells will be extensively tested *in vitro* and in human gene transfer experiments. Clinical grade supernatants will be screened for contaminating microorganisms, including mycoplasma, and for endotoxin. The S^+L^- assay is used for detection of replication competent virus and virus supernatants following amplification on 3T3 cells. Before utilization in human studies, the cells will be certified to be free of contaminating replication competent retrovirus and other adventitious agents by criteria recommended by the FDA. All batches of the LNs105 producer cell lines tested in humans will have met FDA specifications for current clinical human gene therapy trials in progress. See Appendix D for FDA regulations (CBER "Points to Consider" in Human Somatic Cell Therapy and Gene Therapy).

F. Justification for Proceeding to a Human Trial

We are proceeding to this pilot gene therapy study for numerous reasons that include: 1) the pressing need for therapeutic advances, 2) the fact that pharmacological therapy with presently available anti-retroviral agents such as nucleoside reverse transcriptase inhibitors (AZT, ddI, ddC) have proven toxic and of very limited efficacy (13), 3) the long-term benefits that gene therapy potentially offers for HIV-1 treatment, 4) the availability of a retroviral vector and packaging line previously tested to be safe in humans (70–76), and 5) lack of a good animal model for HIV-1 pathogenesis (and hence for *in vivo* testing of therapies).

G. Previous Human Gene Therapy Trials

We have designed this human trial with the benefit of a great wealth of data acquired in the past three to five years by other groups of investigators that support not only the general safety of retroviral vector-based gene therapy in humans, but also the particular vector and packaging system we will use (70–73). Most human gene transfer trials approved by the RAC and all of its subcommittees to date have employed retroviral vectors for *ex vivo* gene transfer (77,78). LN, the vector we are employing to deliver the intracellular antibody gene, was used in the first human gene transfer trial (71) to transfer the NeoR marker gene to tumor-infiltrating lymphocytes. The first actual human gene therapy trial (i.e., not solely a gene-marking trial) began in September, 1990 and involved retroviral vector-mediated transfer (by LASN) of the adenosine deaminase (ADA) gene into the lymphocytes of a patient with ADA deficiency (78). Toxicity referable to the vector or the packaging line was searched for and was not observed in these and subsequent trials (77,78).

Furthermore, the majority of approved studies have used the packaging cell line we will use, PA317 (70–73). In the human trials to date, none of the production batches of retroviral vectors that have been used in patients have tested positive for replication-competent helper virus (78). We are aware that thymic lymphoma has developed in three of eight rhesus monkeys given bone marrow infected with a retroviral stock known to be heavily contaminated with replication-competent virus (78). Although the disease observed in these monkeys was not observed in previous studies of infection of cynomologous macaque and rhesus monkeys with amphotropic murine helper virus (79,80), a broad consensus exists that stringent testing for helper virus is necessary for human trials (77,78), and we plan the comprehensive studies now standard in gene therapy protocols.

Three other trials approved for treatment of HIV-1 disease have guided our preparation of this proposal. One is a marker study involving retroviral vector-mediated transfer of a marker gene to HIV antigen specific T cells (81). Another study is a protocol involving retroviral vector transfer of a trans-dominant *rev* protein to peripheral blood lymphocytes of HIV-1 infected patients (G. Nabel, personal communication). A third study is a protocol involving retroviral vector transfer of a ribozyme gene that cleaves HIV-1 RNA (F. Wong-Staal, personal communication).

We therefore believe that these considerations and the potent anti-viral effects we have observed in tissue culture T cell lines provide ample justification for this pilot study to test the safety and effects of this intracellular anti-envelope single chain antibody in human subjects with HIV-1 infection.

2.0 Objectives

The principal objectives of this feasibility study of somatic cell gene transfer into HIV-1-infected humans are:

a. To evaluate the safety of infusing autologous lymphocytes that have been transduced *ex vivo* with a retroviral vector encoding a human sFv105 intrabody against HIV-1 envelope protein.
b. To compare *in vivo* in each patient the kinetics and survival of sFv105-transduced cells with a separate aliquot of cells transduced with a control vector (identical except for the sFv105 cassette).
c. To evaluate the *in vivo* expression of sFv105 in transduced lymphocytes.
d. To investigate whether host immune responses directed against the transduced cells will occur *in vivo*.
e. To make preliminary observations on the effects of gene therapy with the sFv105 single chain antibody on *in vivo* viral mRNA expression, viral burden, and $CD4^+$ lymphocyte levels.

3.0 Patient Selection

Six patients with Human Immunodeficiency Virus-1 (HIV-1) infection will be enrolled. The subjects will be recruited through individual physician-patient encounters in the Harvard Medical School Infectious Disease Clinics (MGH, BWH, BI and NEDH). No written advertising is planned. The patients will see their usual caregiver throughout the study and communication with that caregiver is planned. The period of surveillance is one year. After that, follow-up for research purposes will be carried out as indicated by the results of the trial. No extra costs to the patients will occur.

The following eligibility criteria will be used:

3.1 Inclusion Criteria

3.11 HIV-1 infection documented by a Federally licensed positive ELISA Assay and confirmatory Western blot analysis.

3.12 Have $CD4^+$ T cell levels $>250/\mu l$ or >15–29% and have a positive DTH skin test and reside in CDC HIV classification categories A1 or A2 (see CDC HIV Classification from MMWR of 12/18/92 included in Appendix B) (82).

3.13 Adequate hepatic function:
e.g., bilirubin $<1/5$ mg^2, SGOT <60 iu.

3.14 Adequate renal function:
BUN <20 mg^2, Serum creatinine <1.5 mg/dl.

3.15 Adequate bone marrow function:
e.g., WBC > 4000/mm^2, Platelets > 100,000/mm^2.

3.16 Prior therapy requirements and allowances—none.

3.17a Age ≥18 and ≤ physiological 65 years.

3.17b Patients of either sex are eligible. All patients with reproductive potential, male or female, must agree to use an effective method of contraception during the study period. Female subjects must have a negative serum pregnancy test.

3.17c Karnofsky score >80% at enrollment.

3.18 The patients may take zidovudine (AZT) or other nucleoside analogue reverse transcriptase inhibitor (ddI, ddC) since this is one of the treatment options that was recommended from the 1993 State-Of-The-Art Conference on anti-retroviral therapy for patients who recide in CDC HIV category A2 (see Appendix B) (13). It is understood that AZT intolerance (resulting from toxicities including, but not limited to, bone marrow suppression, intractable nausea, myopathy) could occur on study in any given patient and necessitate withdrawal of the drug.

3.19 The patient must be able to provide informed consent.

3.2 Exclusion Criteria:

a. Laboratory parameters:
   i. $CD4^+$ cell count <250 cells/mm^3. This baseline CD4 level will be established by two or more measurements at least two weeks apart.
   ii. Hematocrit <30%.
   iii. Platelet count <100,000/mm^3.
   iv. White blood cell count <4000/mm^3.
   v. Absolute neutrophil count <1000/mm^3.
   vi. Creatinine >1.5 mg/dl.
   vii. Total bilirubin >1.5 mg/dl.
   vii. SGOT >60 iu.
b. Current or previous AIDS (Acquired Immunodeficiency Syndrome)—defining opportunistic infection (revised 1993 CDC criteria, Appendix B & C), Kaposi's Sarcoma, lymphoma, cervical or other carcinoma, or hairy leukoplakia.
c. Pregnant or nursing women.
d. An acute infectious illness within one month of initiation of treatment.
e. Any other serious chronic illness including, but not limited to: diabetes, chronic active hepatitis, sarcoidosis, active autoimmune disease (such as rheumatoid arthritis, systemic lupus erythematosus, Reiter's Syndrome, inflammatory bowel disease, thyroiditis, etc.), coronary artery disease, cardiomyopathy, chronic obstructive pulmonary disease, dementia.
f. Patients receiving systemic glucocorticosteroids.
g. Allergy to penicillin and synthetic derivatives, streptomycin or amphotericin B.
h. Active alcohol abuse or drug abuse or psychiatric impairment or occupational or personal circumstances that in the opinion of the investigators would prevent compliance with the protocol.
i. Patients who have undergone alternative non-FDA approved treatment within four weeks of initiation of the protocol.

4.0 Patient Entry

a. Enrollment:

Subjects who meet the eligibility requirements will have the study explained in detail to them by Dr. Wayne Marasco or one of his clinical colleagues (designated at points in the protocol as investigating physicians). Subjects will then be asked to give witnessed, informed consent in writing.

b. Pretreatment evaluations:
   i. Complete medical history, complete physical examination, and performance status assessed by a study physician.
   ii. CDC, differential count, platelet count, PT, PTT.
   iii. Chemistries: glucose, calcium, total protein, albumin, uric acid, phosphate.
   iv. BUN, creatinine.
   v. Urinalysis
   vi. Pregnancy test (where indicated).
   vii. Electrocardiogram, chest X-ray.
   viii. p24 antigen level.
   ix. CD4 counts.
   x. Quantitative PCR for viral load RNA synthesis.
   xi. Cryoprecipitation of 10 ml of serum and PBMC derived from 45 ml of peripheral blood.

Patients registered to this protocol will contact the Quality Control Center (QCC), J810, (617) 632-3761, FAX: (617) 632-2295. The QCC asks for the following information:
Patient name and telephone number
Protocol name and number
Date treatment begins
Patient name
Date of birth
Service
Patient ID number
Primary physician
Primary treatment institution
Confirmation of eligibility (check list when applicable)

5.0 Treatment Programs

Gene Transfer Methods

In the gene transfer procedures outlined below, measures will be employed to avoid activation of latent HIV-1 present in patient peripheral blood lymphocytes. The non-nucleoside reverse transcriptase (RT) inhibitor nevirapine and CD4-*Pseudomonas* exotoxin [CD4(178)-PE40], will be included during the *ex vivo* expansion and transduction of patient lymphocytes. The latter agent is a fusion protein consisting of the HIV-1 envelope binding region of human CD4 linked to the translocation and ADP-ribosylation domains of *Pseudomonas aeruginosa* exotoxin A (83). Binding of one toxin domain allows entry of the lethal second domain into the cell: CD4(178)-PE40 has been shown to selectively eliminate infectious HIV-1 from cultures of human T cell lines when used in concert with reverse transcriptase inhibitors (84). Nevirapine belongs to the non-nucleoside RT inhibitors, a group of structurally diverse compounds that non-competitively inhibit HIV-1 RT and viral replication at nM concentrations, typically having therapeutic indices (assayed in cultured cells) of 1000 to 1, and are highly specific for HIV-1, lacking activity against HIV-2, SIV, or murine reverse transcriptases (85–87). This synergistic combination of an agent selectively virustatic for HIV-1 and an agent selectively cidal for gp120 expression cells will prevent amplification of HIV-1 during the protocol without interfering with transduction by the PA317-packaging vector. Nevirapine and CD4(178)-PE40 have been approved by RAC for prior HIV gene therapy protocols. Finally, we will assay for HIV-1 gag expression in the expanded cells by a commonly used and highly sensitive (pg/ml range) HIV-1 p24 antigen capture assay (DuPont-NEN). Other novel reverse-transcriptase inhibitors and antivirals (85,88) have potential application to this problem in future protocols.

5.1 Drug Formulation and Procurement

a. Harvesting of Patient PBMC for Ex Vivo Stimulation

All materials will be handled within the BL2+ containment suite in Jimmy Fund, 7th floor. Containment room 720D will be exclusively used for this clinical protocol. A chain of purity and sterility will be maintained and documented. As the procedures of cell procurement, purification, activation, transduction and expansion will take approximately two-to-three weeks, we plan on performing studies on one patient at a time at approximately one month intervals.

Following completion of baseline data acquisition, study subjects will undergo one cycle of lymphopheresis in the DFCI blood bank or other designated clinic facility. Fresh peripheral blood mononuclear cells will be separated from erythrocytes and neutrophils by Ficoll-Hypaque density gradient centrifugation. The PBMCs will then be washed, and depleted of $CD8^+$ cells utilizing murine anti-human CD8-monoclonal antibody coated flasks (Applied Immune Sciences CELLectorTM Flasks). Each flask will be loaded with 30 mls of $2–3 \times 10^6$ cells/ml and incubated at room temperature for one hour. Non-adherent cells are to be stimulated in CM with 10 ng/ml OKT3 antibody [CM = AIM-V serum-free media (Gibco) with 2 mM glutamine, 50 U/ml penicillin, 50 μg/ml streptomycin, 2.5 μg/ml Fungizone, IL-2 1000 U/ml and the HIV-1 reverse transcriptase inhibitor nevirapine (Boehringer-Ingelheim) at 10 μg/ml and CD4(178)-PE40 (Upjohn) at 50 nM [nevirapine and CD4(178)-PE40 will be supplied as free agents]. Cellular phenotype is assessed by flow cytometry prior to expansion. OKT3 will be stopped after this initial round of stimulation to prevent significant carry-over into the patient.

b. Transduction.

OKT3-activated cells are resuspended in complete fresh CM (above) except with 100 U/ml of IL-2 (Cetus, Emeryville, CA) at $1–2 \times 10^6$ cells/ml. Two identical aliquots of cells will be transduced simultaneously, one with the sFv105 encoding vector and with the control vector LN (vector lacking the sFv105 cassette). Frozen viral supernatants will be stored at −80°C. On the day of transduction, aliquots will be thawed and passed through 0.45 micron filters. Transduction will be performed by adding to the culture medium equal volume of viral supernatant (MOI of 1) supplemented with protamine sulfate (89) at a final concentration of 8 μg/ml. After overnight incubation at 37°C, the cells will be washed in CM, resuspended at $1–2 \times 10^6$ cells/ml and transduction will be repeated. After an additional 24 hr, the transduction will be repeated a third time using identical conditions. After the final transduction, cells will be washed 3 times in CM and introduced into tissue culture bags for large scale expansion for therapy.

c. Expansion in IL-2 and Selection of Transduced Lymphocytes

Transduced lymphocytes, resuspended at $1–2 \times 10^5$ cells/ml in complete fresh CM containing 100 U/ml of IL-2 and 500

μg/ml G418, will be expanded in 3000 ml culture bags each containing about 500–1000 ml media. Cells are grown to maximum density (about $1–2 \times 10^6$ cells/ml). Expansion is estimated to take 7–8 days. Efficiency of selection will be monitored in parallel by viability of non-transduced cultures. After selection is complete, G418 will be removed and the cultures expanded up to 1×10^9/kg for reinfusion. Nevirapine and CD4(178)-PE40 will be stopped at completion of the expansion. The subsequent washings will prevent significant carryover of these two agents into the patient.

A p24 antigen capture and reverse transcriptase assay (RT) will be done at completion of this stage and again within a few days of reinfusion. Testing on the final cell pellet prior to reinfusion will not be possible in all cases since these cells must be expeditiously transferred into the patient or they will die.

5.2 Description of Treatment Protocol

Infusion of Transduced, Expanded Autologous Lymphocytes

The transduced cells will be harvested, washed, and resuspended in sterile PBS. The final cell preparation will be filtered through a platelet filter and transferred into a syringe or transfusion pack for infusion. Patients will be admitted to the Dana-Farber Cancer Institute. Interim history and physical examination will be performed by an investigating physician, intravenous catheterization with standard sterile technique will be performed and good venous return and absence of infiltration will be verified immediately before infusion. The infusions will be performed with Dr. Marasco or one of the investigating physicians present. The infusion will be of not less than 5×10^8 cells/kg (and not more than 1×10^9 cells/kg) of body weight. (The dose target per infusion in the ADA Clinical protocol (90) was $1–3 \times 10^9$ per kg). Total volume of infused cells should not exceed 10 ml/kg of body weight. After an initial test infusion of 1–5% of the total volume, cells will be infused over the next 60–120 minutes. During infusion, the cell suspension will be mixed gently approximately every 5 minutes while the patient is being observed for acute and subacute toxicity. Vital signs will be monitored before infusion and every 15 minutes during and 2 hours after infusion or until the patient is stable. The infusion will be terminated if systolic blood pressure falls below 80 mm Hg, oxygen saturation falls below 90%, or other evidence of major systemic toxicity occurs. Patients will have nursing observation for 24 hours after infusion; patients will be discharged after 24 hours if there are no complications. A blood sample or samples will be drawn prior to discharge for initial studies detailed in the following section.

5.3 Adverse Effects

5.31 Risks Related to Harvesting Cells and Reinfusion

The risks of venipuncture include bruising and minor bleeding. The amount of blood that will be phlebotomized or obtianed by lymphophoresis should not cause volume depletion hemodynamically significant enough to result in syncope or to significantly exacerbate symptoms of mild chronic anemia attendant to HIV-1 infection (initial eligibility hemocrat cut-off is 30%). Nevertheless, patients will be observed for at least one hour after phlebotomy and examined for clinically significant orthostatic blood pressure changes.

Since harvested patient cells will undergo expansion and manipulation *in vitro*, there is a risk of microbial contamination of bacteria, mycoplasma, fungi, or viruses. All procedures will be optimized to maintain sterility of patient cells. Monitoring of cells prior to reinfusion will include: viability (at least 80% trypan blue exclusion), culture for helper virus as indicated above, surveillance for aerobic and anaerobic bacterial and fungal contamination by culture in thioglycolite broth, on blood agar and chocolate agar and gram stain. A probe hybridization test will be used to test for mycoplasma and other prokaryotic organisms. The standard limulus assay is planned to test for endotoxin. A gram's stain of the final cell pellet will be done prior to infusion to confirm the absence of organisms. Finally, to ensure that all components of the protocol are in place, we plan to dry-run the protocol at least once on human PBLs before the first patients are treated and once on HIV-1-infected human PBLs to ensure that suppression of HIV-1 replication can be accomplished and that adequate numbers of $CD4^+$ T cells can be harvested, transduced and expanded. We will submit these data on full scale "dry run" experiments including details of the testing for efficiency of transduction and assays for p24, RCR, and microbial contaminants to the SRC, IRB and IBC for review prior to infusion of transduced cells into patients.

5.32 Toxicity Criteria and Reporting of Adverse Effects

5.32a Monitoring of Adverse Effects

Subjects will be clinically evaluated at different intervals as determined above (Project protocol). All side effects will be graded using standard criteria (see attached). If one patient experiences recurrent grade 3 or any grade 4 toxicity, that patient will be removed from study and the sample size increased to 5 cases. If two of 4 cases experience this level of toxicity, the study will be suspended.

Other toxicities/treatment complications that will be monitored and if observed in any two patients will result in suspension of the trial will include:

1. Greater than 10-fold rise in p24 levels.
2. 4-fold increase in CTL activity against the transduced cells.
3. Greater than 50% decline in CD4 counts or rapidly declining levels of CD4 cells (to be defined).
4. Failure to detect transduced cells (sFv105 and vector) by limited dilution DNA PCR during the first clinic visit (see Table 1).

5.32b Reporting of Adverse Effects

Adverse reactions will be reported to the DFCI Human Subjects Committee in writing within ten working days and to Investigational Drug Branch, P.O. Box 30012, Bethesda, MD 20824, Tel. (301) 496-7957, and include all fatal events, all life threatening events (grade 4) which may be due to drug administration or to the first occurrence of any previously unknown clinical event (regardless of grade).

5.4 Therapy Duration

The period of study surveillance is one year. After that year, follow-up for research purposes will be carried out as indicated

by the results of the trial. No extra cost to the patients will occur. The patients will see their usual care giver throughout the study, and communication with that care giver is planned.

6.0 Federal Reporting Requirements for Adverse Drug Reactions

The Food and Drug Administration requires that all adverse reactions occurring during treatment with an investigational drug be reported as follows. FDA Form 3500A may be used.

Report by phone by the P.I. to the Dana-Farber Cancer Institute Protocol Administration Office and to Dale Spriggs, Ph.D., Director of Clinical Studies at Virus Research Institute (the sponsor of this study) **within 24 hours.**

1. All life-threatening events (grade 4)
2. All fatal events while on study
3. First occurrence of any toxicity regardless of grade.

Written report must follow **within 10 days** to:
Dale Spriggs, Ph.D.
Director of Clinical Studies
Virus Research Institute

A copy of all written reports will be sent to the Protocol Administration Office as they are sent to the Sponsor.

7.0 Required Data

The patients in this study will have direct access to an investigating physician via telephone or pager. The following patient visits and data collection are planned (on the schedule indicated in Table 1 below):

1. Interim history and physical examination by an investigating physician.
2. Isolation of PBLs for:
   a. Limiting dilution quantitation and DNA PCR to assess transduced cell survival *in vivo*. PCR primers will be chosen to achieve amplification specific for the sFv105 vector and control vector. The longevity of the transduced cells, the primary experimental variable to be monitored in this trial, is difficult to prospectively estimate. Nevertheless, we note that in the ADA trial a larger number of gene-corrected T cells survived in the first patient for over six months after cell infusions were stopped, a result that confounded pretrial concerns that cells would have a severely short lifespan (78).
   b. RNA PCR to assess sFv105 expression *in vivo*.
   c. CTL assay—To perform this analysis, a blood sample will be obtained prior to treatment to derive lymphocytes which will be immortalized using the Epstein-Barr virus. An aliquot of these cells will be further infected with either control vector or sFv105 vector and selected for G418 resistance. Expression of Neo® or sFv105 will be confirmed by RNA analysis. These cells will subsequently be used in the laboratory as target cells for the cytolytic T cell assay (^{51}Cr release assay). PBMCs will be expanded for seven days *in vitro* in the presence of recipients transduced and EBV immortalized cells. At no time will these cells be brought into the same building where the patient or their cells are being treated.
   d. Virus load (RT-PCR).
   e. Phenotype of transduced cells *in vivo* by FACS analysis and PCR.
3. CD4 count
4. p24 antigen level
5. Complete blood counts and standard chemistries (SMA-20)
6. Collect serum and PBL's for archiving

Note: The volume of blood to be drawn during the clinic visits will range from 50 to 100 ml (see Table 1).

7.2 Data Collection

All data will be collected by the investigating physicians, or where applicable by the co-investigators at VRI. We will consult the Quality Control Center on forms and data collection methods. The forms will include:

TABLE 1

		Day				*Week*						
	Pre-Study	*1*	*7*	*14*	*28*	*8*	*12*	*16*	*20*	*24*	*36*	*52*
Test												
*1.	X	X	X	X	X	X	X	X	X	X	X	X
2-a.	X	X†	X		X		X		X		X	X
2-b.	X		X		X		X		X		X	X
2-c.							X					X
2-d.	X				X		X				X	X
2-e.	X						X					X
3.	X				X		X		X	X		X
4.	X				X		X					X
5.	X	X	X	X	X	X	X	X	X	X	X	X
6.	X				X	X	X	X	X	X	X	X

*Numbers refer to designations in immediate preceding section.
†An additional sample will be taken several months after injection to examine for the presence of DNA in circulatory PBL.

FORM	*SUBMISSION TIME*
On study	Within 1 month of patient entry
Summary and Evaluation	Within 1 month of completion of therapy

8.0 Modality Review

Non-Applicable

9.0 Statistical Considerations

9.1 The primary endpoints of this feasibility study are to investigate the toxicity and *in vivo* and *in vitro* effects of gene transfer in HIV-1 infected patients. If five cases are treated and if the true underlying rate of limiting toxicity (defined in 5.32a) is 20%, then there is a 74% chance that there will be no episodes seen in 4 cases or only 1 in 5. If, on the other hand, the true underlying rate of limiting toxicity is 60%, then there is only a 7% chance that we will see minimal (0/4 or 1/5) toxicity and fail to see evidence of this high rate.

9.2 Data collected during this feasibility study will include the following variables: clinical history and physical examination parameters, vital signs, report of adverse events, chemistry profile, CBC and differential, platelet count, urinalysis, $CD4^+$ T cell count, HIV-1 antibody titer, leukocyte viral load by PCR and CTL response. These variables will be measured at multiple time periods. Findings from this study will be tabulated and presented, and results will be analyzed. All out of range laboratory data will be addressed to resolve the discrepancy such as repeating the assay.

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11.0 Informed Consent

The investigating physicians will carefully explain the study's goals, risks, potential discomforts, and limitations to each perspective subject. After the patient has had at least one week to read the consent form, ask questions of the study physicians and consult his or her family, other physicians and sources of advice as he or she wishes, a signed, witnessed consent form will be obtained by the investigating physician. Recruitment of subjects will be through referrals based on individual physician-patient encounters in the Harvard infectious disease system. No written advertising for patients is contemplated. The consent document includes a request for autopsy at the time of the patient's death.

12.0 Appendices

Appendix A:	ACTG Toxicity Scale
Appendix B:	CDC HIV-1 Classification (MMWR 12/18/92)
Appendix C:	Conditions included in the 1993 AIDS surveillance case definition
Appendix D:	CBER "Points to Consider" in Human Somatic Cell Therapy and Gene Therapy
Appendix E:	Published Manuscripts
Appendix F:	Safety Protocols (Majenta Corporation)

DANA-FARBER CANCER INSTITUTE

INFORMED CONSENT
FOR RESEARCH

Issue Date: 8/31/94

PROTOCOL NUMBER & TITLE: 94-049 sFv 105

SUBJECT/PATIENT NAME: _____ **DFCI I.D. NUMBER:** _____

INTRACELLULAR ANTIBODIES AGAINST HIV-1 ENVELOPE PROTEIN FOR AIDS GENE THERAPY

Dr. Wayne Marasco and his colleagues are conducting a research study to find out more about gene therapy for the treatment of HIV and AIDS (Acquired Immunodeficiency Syndrome). You are being invited to participate because you have an infection with the Human Immunodeficiency Virus (HIV-1). Six patients will participate in the study. The purpose of this study is to test the safety and effects of an investigational treatment for HIV-1. The treatment is called "Intracellular Antibody Gene Therapy."

This therapy is called "gene therapy" because the object of it is to give your white blood cells a new gene, the gene for a human intracellular antibody. Antibodies are proteins produced by your immune system that have the ability to bind infectious agents like bacteria and viruses and clear them from your body. The antibody that will be used to treat your cells has been engineered so that the protein is produced inside your $CD4^+$ T cells, a type of white blood cell that becomes infected with the AIDS virus. The antibody is directed against a critical protein of the AIDS virus, the envelope glycoprotein gp120. This viral protein is both directly responsible for killing infected white blood cells and is also responsible for the immune system destruction of your white blood cells. We hope that the treated white blood cells will become protected from HIV infection when they are back in your body because the antibody may protect infected white blood cells and may lead to the production of less infectious viruses.

Tests we have done with human cells in the laboratory has shown that the intracellular antibody does help to protect human cells growing in laboratory dishes from HIV-1. In this study, we will study what happens to you and your white blood cells when they are put back into your body after being given the gene for the intracellular antibody. That way we will find out whether what works in white blood cells in laboratory dishes will work in white blood cells when they are in your body. We will find out if this can be done safely and whether or not there are any side effects. Any significant new findings from this study will be made known to you.

If you agree to participate in the study, the following is what the therapy will entail:

1. One of the investigating physicians will take your complete medical history and perform a complete medical examination.
2. You will have blood tests and X-rays to determine if you are able to complete the study.
3. If you are eligible for the study, you will come into the clinic and will have a needle (an intravenous line or "I.V.") inserted into your vein to remove some of your white blood cells. To do this, the I.V. will be connected to a machine which will remove some of your white blood cells while returning to you your plasma and red blood cells. This procedure is called "lymphophoresis." We will watch you for an hour or so and make sure you tolerate the blood drawing procedure without any problems and, if so, you will then be able to go home on the same day.
4. In the laboratory, some of your white blood cells, your T cells, will be separated from the rest of your white blood cells and placed in laboratory dishes. There they will be treated in various ways to make them grow and to keep any HIV in them from spreading in the dishes. After the white blood cells have multiplied in number, they will be treated with the intracellular antibody.
5. While your white blood cells are being given the new gene in the laboratory and allowed to multiply (two to three weeks), you will be kept informed regularly about the schedule of what needs to be done next. You will be notified when the cells are ready to be put back into you. You will be admitted to the Dana-Farber Cancer Institute, and a needle will be inserted into your vein. Your genetically modified and expanded white blood cells will then be infused back into you through the needle over a period of a few hours.
6. Follow-up Studies. Because this is a new and investigational treatment, we will be observing you closely during and after the treatment to determine if there are any side effects to the therapy. We will also closely monitor the effects of this treatment on your immune system. We will do this through a combination of regularly talking to you about any symptoms or problems you may have, examining you in the clinic, and by doing a variety of blood tests. It will take about one year of such visits to complete your participation in the study, at first every week or so for a month, and then about every month or so. Small amounts of blood for testing will be drawn at each visit and will range from about four to ten tablespoons.

7. Although the study will be completed in about one year, follow-up will be life-long. If you move, you should provide Dr. Marasco or one of his colleagues with your new address.

Possible Risks, Discomforts, and Side Effects

Participation in this study may involve some added risks or discomforts. These include:

1. You may experience discomfort or bruising from blood drawing and intravenous infusions. Sometimes people get lightheaded or even faint after blood drawing. There is also the remote possibility of infection at the blood drawing site or I.V. site.
2. Although the DNA that will be inserted into your white blood cells is considered safe, there is a remote chance that inserting the gene into the DNA of your cells could cause cancer to develop, which would create additional problems and may require additional treatments. The development of cancer could also be fatal. Laboratory studies and previous studies of similar gene therapy in people suggest that this possibility is very low. However, this is a new procedure and we do not know whether the white blood cells could become abnormal after a long period of time.
3. Although the intracellular antibody has shown protection of human cells from HIV in the laboratory, it is possible that this therapy could increase the amount of virus in your blood as well as decrease your survival.
4. The inserted DNA has a gene that can cause a few antibiotics to be inactivated in the test tube. This gene will not likely be active in any appreciable amount in your body and many other antibiotics that are not inactivated by it will be available for treating any potential bacterial infections. Therefore it is unlikely to significantly interfere with giving you effective antibiotics for any infections you may develop in the future.
5. Although many efforts will be made to ensure no bacteria, viruses or other kinds of infections get into your white blood cells while they are being genetically modified outside your body, a small possibility exists that such contamination could occur and cause harmful or even fatal infection when the white blood cells are put back.
6. Although previous animal and human studies suggest it is unlikely, there is a possibility that having a new gene in some white blood cells of your body could provoke a response by your immune system against those and other cells in your body.

Your participation in this study is entirely voluntary. You may refuse to participate or withdraw from the study at any time and for any reason without jeopardizing your future health care in any way at this institution.

Because only a relatively small number of white blood cells compared to the total number of cells in your body will be transferred back to you in this study, it is not known if you yourself will benefit. However, the knowledge gained from this study may benefit others in the future.

There will be no added costs to patients participating in this study. This study will not pay for routine medical costs.

Alternative Therapies

Neither HIV infection nor AIDS can be cured with presently known treatments. Alternative treatments available to you that may have some beneficial effects are medicines such as zidovudine (AZT), didanosine (ddI), and zalcitabine (ddC). Medicines (such as antibiotics), surgery or radiation therapy can be used to treat some complications of HIV infection. All of the above treatments, however, can and will be used if they are indicated for you according to standard clinical practice during this study—they will not interfere with intracellular antibody gene therapy. In other words, intracellular antibody gene therapy will be used in addition to these other established methods of treatment. You should also know that other investigational treatments for HIV are being investigated elsewhere and you can be referred to physicians who are conducting such trials. At some late time, should new alternatives for treatment of HIV become available, they will be discussed with you and offered to you. You also have the option to receive no treatment at this time.

Confidentiality

The sponsor of this study, Virus Research Institute, may have access to your medical record, including sensitive information which it may contain. Sensitive information, as defined by law, includes information such as HIV test results, communications with social workers and psychotherapists, and substance abuse. Every attempt will be made to preserve strict confidentiality and privacy throughout the study on any information that is collected. When results of a study like this are reported in medical journals or at meetings, the patients taking part are never identified. Research records will be kept confidential to the extent provided by law and are made available for review by the Food and Drug Administration or other authorized users, only under the guide-

lines established by the Federal Privacy Act. A qualified representative of the National Institutes of Health may inspect patient and study reports.

Avoiding Pregnancy and HIV Transmission

If you are female and capable of child-bearing, a sample of urine will be collected before the study is begun in order to be as sure as possible that you are not pregnant. Both men and women who participate must agree to use an effective contraception method (such as condoms, the pill, diaphragm, or interuterine device) to prevent reproduction for the duration of the study since it is possible that the treatment being tested could cause harm to an unborn child. Whether or not they also use a method such as the pill or diaphragm to ensure prevention of pregnancy, all people with HIV infection should always use a condom during sex to prevent transmission of HIV.

Request for Autopsy

If you agree to participate in this study, if you should die, an autopsy will be requested of your family. An autopsy is important even if death was accidental and not caused by illness or related in any way to the gene treatment, since much information that is of potential benefit to future recipients of gene therapy can be learned that way. We ask that you let your family know that you wish to have an autopsy. Although we are requesting an autopsy, it will not be required to participate in this study.

Laboratory Research on Human Tissue

Occasionally, laboratory research on human tissue does result in discoveries that cause the basis for new research products or diagnostic and therapeutic agents. We ask that you make a gift to the Institute of any rights you may have to the proceeds from such discoveries.

If I have other questions or research related problems I may call Dr. Wayne Marasco at (617) 632-2153 or through the DFCI page operator at (617) 632-3352, beeper 1561.

Dr. _____ has explained the purpose and risks of this study to me and answered all of my questions.

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HOW MUCH HAS CHANGED AND HOW LITTLE HAS CHANGED

This protocol is being published for its historical significance, having been the first intracellular antibody (intrabody) gene therapy protocol to be approved by the RAC (in June 1995). I re-read the protocol with interest and quickly realized how much has changed and how little has changed.

We abandoned our efforts to take this protocol into the clinic shortly after determining that intrabodies against other HIV-1 target proteins, particularly HIV-1 Tat were more potent inhibitors of HIV-1 replication than the anti-gp 120 intrabody sFv105 (Mhashilkar *et al.*, 1995, 1997; Poznansky *et al.*, 1998). This was established by transduction of $CD4^+$-selected PBMCs from HIV-1-infected individuals at different stages of disease with a direct comparison of the antiviral effect of sFv105 versus sFvtat1Ck (Poznansky *et al.*, 1998). More recent studies confirm the breadth of the anti-tat sFv intrabody inhibition through dose challenge experiments with a panel of primary HIV-1 isolates (submitted for publication). A new clinical gene therapy trial using a completely humanized version of murine sFvtat1Ck termed sFvhutat2 is scheduled to begin in late 1998.

After a fresh reading of this clinical protocol, several sections that discuss antiretroviral therapies seemed particularly suitable for comment, due to the rapid changes that have occurred in the area of HIV-1 therapeutics. Recent improvements in anti-retroviral drug treatments, particularly highly active antiretroviral therapies (HAART) have resulted in remarkable reductions of viral loads in HIV-1 seropositive patients and have become standard-of-care (Carpenter *et al.*, 1997; Feinberg *et al.*, 1998). However, there are limitations to the therapy, particularly in patients who have been on multiple mono-therapies in the past and/or in patients who have started HAART later in the course of their HIV-1-infection. In these patients, the antiretroviral responses have been poor (Collier *et al.*, 1996; Gulick *et al.*, 1997; Hammer *et al.*, 1997). In addition, strict compliance with HAART therapy is required to maintain long term suppression of HIV-1 replication with viral rebound occurring even with short drug holidays

(Dr. Martin Hirsch, MGH, Boston, MA, personal communication). Furthermore, a reservoir of latently infected cells persists in these HIV-1-infected individuals (Wong *et al.*, 1997; Finzi *et al.*, 1997). These limitations combined with the lifelong adherence that is probably necessary to maintain suppression of viral replication underscores the need for additional therapeutic approaches to enhance immune function against HIV-1. As a result, gene therapy for the treatment of HIV-1 infection and AIDS has captured the renewed interest of a number of clinical investigators as an attractive addition to conventional pharmacologic therapies. Choosing the population of HIV-1-infected patients to study in the ever-changing arena of HAART is and will remain a formidable challenge.

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