HUMAN GENE THERAPY
FROM PROMISE TO PRACTICE

Randall W. Moreadith, MD, PhD

June 17, 1993

INTRODUCTION

As I reviewed the literature on the subject of human gene therapy, several aspects of its evolution seemed particularly noteworthy. What began initially as an ethical debate, growing out of several very significant scientific events in the late 1960s and early 1970s, soon became the topic of heated exchanges between scientists, theologians, "public defenders" and eventually the government. These debates set the stage for what is currently one of the most exciting and promising areas of biomedical research.

I have divided today's Grand Rounds into three sections. In the first I will review the development of the field from a historical standpoint. I will then limit the scientific area to somatic cell gene therapy (the issue of germ-line gene therapy will not be discussed). There are currently 42 clinical protocols approved by the RAC; I will discuss three of these in some detail. Two of these protocols have treated patients with rare genetic diseases (adenosine deaminase deficiency and familial hypercholesterolemia) and the initial results are in; the third, cystic fibrosis, is in the very earliest stages of clinical trials. Each of these serves to illustrate some of the promises and difficulties that will need to be addressed with this new technology. And finally, I will discuss some of the future applications of the technology and the impact it will have on the practice of medicine.

GENERAL OUTLINE

I. The Past
   - A. The ethical debate (1967-1989)
     1. threshold
     2. open conflict
     3. extended debate
     4. adaptation

II. The Present
   - A. The beginning - September 14, 1990
     1. ADA deficiency
   - B. Familial Hypercholesterolemia
   - C. Cystic Fibrosis

III. The Future

I. THE PAST

A. The ethical debate (1967-1989)

Threshold - conditions for moral conflict exist

" My guess is that cells will be programmed with synthetic messages within 25 years...The point that deserves special emphasis is that man may be able to program his own cells long before he will be able to assess adequately the long-term consequences of such alterations, long before he will be able to formulate goals, and long before he can resolve the ethical and moral problems which will be raised. When man becomes capable of instructing his own cells, he must refrain from doing so until he has sufficient wisdom to use this knowledge for the benefit of mankind... Decisions concerning the application of knowledge must ultimately be made by society. "

M. Nirenberg, 1967 (1)

Open conflict - leaders or prophetic individuals anticipate ethical problems; significant or notorious cases may epitomize what was predicted; moral convictions collide

The Rogers Case (1970-1973)

In 1969 H. G. Terheggen (a German physician) described two sisters (18 months and 5 years) from a consanguineous marriage with elevated levels of serum arginine (2); their clinical presentation was spastic diplegia, epileptic seizures, and severe mental retardation. Assays of red blood cell arginase revealed a deficiency of the enzyme. In collaboration with Dr. Stanfield Rogers, an Americn scientist from the Oak Ridge National Laboratory working abroad, these children were injected repeatedly during the years 1970-1973 with Shope papilloma virus (SPV is a circular DNA virus which causes warts in rabbits) in the belief that this would lower their serum arginine (it did not work, the formal results were never published). The original observation, made by Dr. Rogers, was that several researchers working with the virus had consistently low serum levels of arginine and that the virus must therefore encode (or induce) the requisite enzymes (it doesn't). Indeed, Dr. R. E. Shope inoculated himself with the virus (without ill effect) resulting in "a lowered serum level of arginine for almost two years". The New York Times (September 20, 1970) ran a lead article entitled "Virus is injected into 2 children in effort to alter chemical traits".

This unprecedented experiment precipitated two major events. The first was a bill introduced in 1971 by Senator Walter Mondale for a "National Commission on Health, Science, and Society" to investigate the legal, social and ethical implications of medical research - including the "aims of geneticists". The second was the first national conference,"The New Genetics and the Future of Man", where

the noted theologian Paul Ramsey and an upstart young scientist W. French Anderson went toe to toe:

> "...untold human suffering, dehumanization, exploitation, radical alteration of the conditions of human existence...laisez-faire system of biomedical investigation" Ramsey, 1971

> "larger question...What about other genes, other viruses? Where does one draw the line? This area holds such promise for alleviating human suffering, and yet is so basic to the needs and emotions of all men, that no individual or group of individuals should take it upon themselves to make the decisions. Only the conscience of an informed society as a whole should make these decisions." Anderson, 1971

Extended debate - social and ethical debate ensues, usually to clarify moral lines; ethical principles are sought, tested and readied to ground and back up the line-drawing

Significant events

Asilomar (1973-1974) self-imposed moratorium on recombinant DNA research (arising out of work by H. Boyer and S. Cohen)

Oversight of recombinant DNA research - NIH took regulatory initiative

1976 - Recombinant DNA Advisory Committee (RAC), guidelines; reports to the Director of NIH, who reports to Congress

Institutional Biosafety Committee / Institutional Review Board

The Cline Case (1979-1980)

In May of 1979 Dr. Martin Cline submitted a protocol to the IRB at UCLA to treat patients with sickle cell disease and other inherited blood disorders (thalassemias) with a "triple combination" recombinant DNA strategy. While still under review, the protocol was altered in September, 1979 to "delink" the plasmid vector to allow "naked" insertion of the globin and herpes virus thymidine kinase genes. He reasoned that the DNA, when introduced into cells, tended to go in in tandem arrays anyway and a functional globin gene would result; this also appeared to remove the requirement for approval by the IBC since the recombinant DNA was no longer "recombinant". The IBC refused to consider the protocol until the IRB ruled on it; the NIH refused to enter the fray until one or the other institutional boards ruled. In July 1980 the IRB disapproved the protocol, agreeing with outside consultants that more animal studies were needed.

Between March and July 1980, Dr. Cline arranged to treat patients in Italy and Israel (Italy had no review committees). The research ethics committee (composed

entirely of physicians) in Israel tried to verify the "delinked" version of the genes as being "nonrecombinant", eventually receiving a telex from UCLA stating the most recent version of Dr. Cline's protocol did not, in fact, involve recombinant DNA molecules. Dr. Cline proceded to inject two patients with the ***original*** (recombinant) DNA molecules...

> "... because I believed that they (the rDNA) would increase the possibility of introducing beta-globin genes that would be functionally effective, and would impose no additional risk to the patient, since it was known that pieces of DNA are efficiently linked in all combinations once they are taken into cells. I made this decision on medical grounds".

Dr. Cline later regretted his decision, admitting he had used "poor judgment". He had violated the ethos of restraint in human gene therapy begun with Nirenberg's appeal, protected by the IRB review, and embodied in the NIH regulations. He was forced to resign his departmental chairmanship and his NIH grants were cancelled; for a period of several years a report of the NIH proceedings on his conduct was attached to all applications for grant support. This event precipitated formation of a select group entitled "The President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research"; their report, *Splicing Life* (1982), was the first report by a government body on the subject of human gene therapy. It proposed the major types of continuing oversight of gene therapy that have since been adopted.

" Once we decide to begin the process of human genetic engineering, there is really no logical place to stop. If diabetes, sickle cell anemia, and cancer are to be cured by altering the genetic makeup of an individual, why not proceed to other "disorders": myopia, color blindness, lefthandedness? Indeed, what is to preclude a society from deciding that a certain skin color is a disorder?"

Jeremy Rifkin, 1983

Significant events

1983 - first packaging cell lines for retroviruses

1984 - creation of Human Gene Therapy Subcommittee

1985 - "Points to Consider" document

A distinction should be drawn between making genetic changes in somatic cells and in germ-line cells. The purpose of somatic cell gene therapy is to treat an individual patient, e.g., by inserting a properly functioning gene into a patient's bone marrow cells *in vitro* and then reintroducing the cells into the patient's body. In germ-line alterations, a specific attempt is made to introduce genetic changes into the germ (reproductive) cells of an individual, with the aim of changing the set of genes passed on to the individual's offspring. The RAC and its working group will

not at present entertain proposals for germ-line alterations but will consider for approval protocols involving somatic cell gene therapy.

Significant events

April 24, 1987 - submission of first gene **therapy** protocol to treat ADA deficiency (the review would subsequently take over three years)

June 10, 1988 - submission of first gene **transfer** protocol

January 19, 1989 - approval of gene **transfer** protocol by Director (NIH) and FDA

January 30, 1989 - Jeremy Rifkin lawsuit; May 15, 1989 - out-of-court settlement of Rifkin lawsuit

May 22, 1989 - first patient received gene **transfer** of modified TILs (tumor infiltrating lymphocytes, marked with the neomycin gene to follow their fate once reinfused into patients with metastatic melanoma).

Adaptation - moral adaptation occurs and public policies are shaped that embody the moral priorities represented by the moral line; all new cases that arise help test and clarify the lines that had been drawn, and lead on to new choices to strengthen or reshape the line.

TABLE 5. ATTITUDES TO GENE THERAPY IN JAPAN AND THE UNITED STATES

Sample	*Public*	*Students*	*School biology teachers*	*Total academic*	*Total scientists*	*United States public (OTA, 1987)*
Number	*532*	*198*	*225*	*706*	*540*	*1273*
Q13. If tests showed that you were likely to get a serious or fatal genetic disease later in life, how willing would you be to undergo therapy to have those genes corrected?						
1. Very willing	25.2	19.7	34.8	23.7	25.4	35
2. Somewhat willing	29.1	31.8	30.4	28.9	28.1	43
3. Somewhat unwilling	18.0	26.8	11.1	15.1	15.6	12
4. Very unwilling	11.7	9.6	10.7	13.4	13.6	9
5. Don't know	16.0	12.1	13.0	18.9	17.3	2
Mean of 1–4	2.19	2.30	1.97	2.22	2.21	1.97
Q14. If you had a child with a usually fatal genetic disease, how willing would you be to have the child undergo therapy to have those genes corrected?						
1. Very willing	36.9	28.4	39.5	30.3	32.1	51
2. Somewhat willing	29.3	37.6	33.2	31.7	30.1	35
3. Somewhat unwilling	11.2	11.3	5.8	9.5	9.7	7
4. Very unwilling	7.0	5.2	8.1	7.5	7.6	4
5. Don't know	15.6	17.5	13.4	21.0	20.5	3
Mean of 1–4	1.86	1.92	1.80	1.93	1.91	1.63

1989 - creation of Biomedical Ethics Advisory Committee, a 14-member public body appointed by the Congressional Biomedical Ethics Board (led initially by Senator Albert Gore), attend RAC meetings

II. THE PRESENT

EDITORIAL
September 14, 1990: The Beginning

On September 14, 1990, at 12:52 pm, a 4-year old girl with adenosine deaminase (ADA) deficiency received an infusion of autologous T cells into which a normal ADA gene had been inserted. The procedure took place in the Pediatric Intensive Care Unit of the Clinical Center of the NIH; she received a billion cells; the infusion took 28 minutes; the entire undertaking was clinically uneventful.

And so began human gene therapy.

Knowledge required for rational use of gene therapy to treat human disease?

Isolate and characterize the gene of interest

Decide on a gene delivery system (How? When? Where?)

Develop a means to control / regulate the expression

A. GENE DELIVERY SYSTEMS

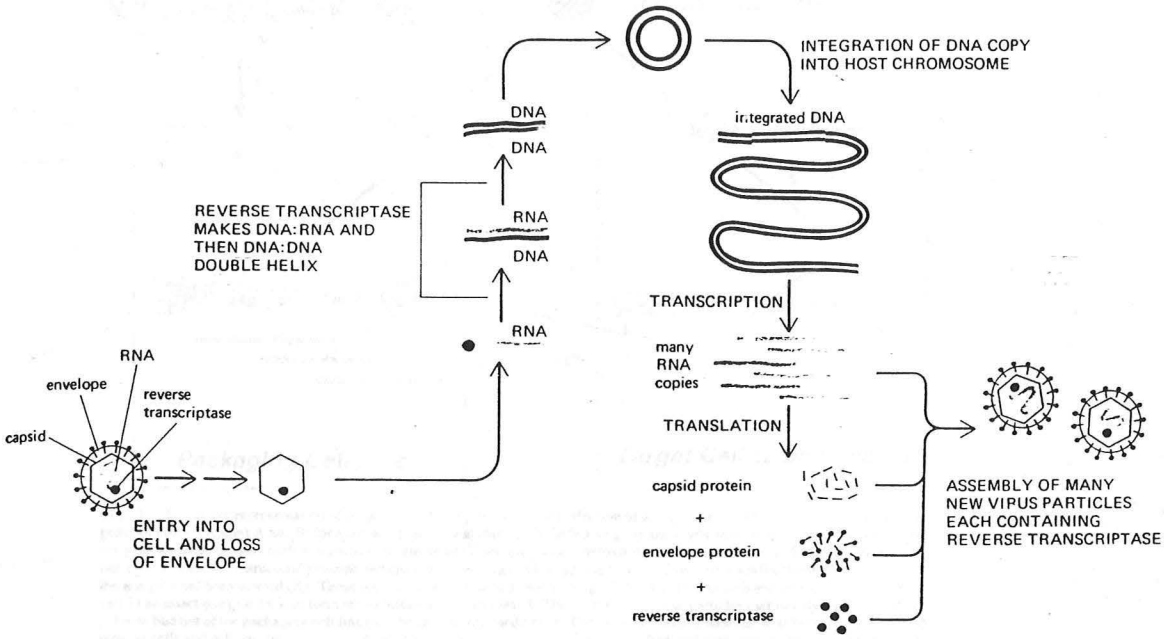
The table below lists some of the methods for deliverying genes into mammalian cells and the likelihood they might be used in humans. Two of these will be discussed in some detail because they have already been approved for use in humans (retroviruses and adenovirus).

Table 1. Methods for delivering genes into mammalian cells and likely applications in gene therapy. (+), Major application; (+/−), some application; (−), little or no application; T, only transient expression; S, stable expression.

Method	Application in gene therapy		Transient (T) or stable (S) expression
	Ex vivo	In vivo	
Viral			
Retrovirus	+	?	S
Adenovirus	+/−	+	T
Adeno-associated virus (AAV)	+	?	S
Herpes virus	+/−	+	?
Vaccinia virus	+/−	+	T
Polio virus	+/−	+	T
Sindbis and other RNA viruses	+/−	+	T
Nonviral			
Ligand-DNA conjugates	−	+	T
Adenovirus-ligand-DNA conjugates	−	+	T
Lipofection	+/−	+	T
Direct injection of DNA	−	+	T
$CaPO_4$ precipitation	+/−	−	S

B. RETROVIRUSES

It is necessary to understand some aspects of the life cycle of retroviruses before we proceed because these properties lend themselves uniquely to applications in humans. Retroviruses (HIV 1 is a retrovirus) are RNA viruses that, upon infection, reverse transcribe their RNA into DNA (hence the name retrovirus) which then integrates into the host genome (typically as a single copy). Following its integration into the genome, multiple copies of RNA are made and packaged following translation of the RNA. This is illustrated schematically in the following figure.



Life cycle of a retrovirus. The retrovirus genome consists of approximately 9,000 bp of an RNA molecule (two per viral particle). It encodes several important proteins which are necessary to complete the life cycle; one, reverse transcriptase, transcribes the RNA into a DNA molecule that is then copied to produce a double-stranded DNA molecule - this inserts itself (with the help of another viral protein) into the host DNA. Transcription and translation of the RNA also generates the proteins required for packaging the mature infectious virions. Retroviruses typically infect only replicating cells.

The great utility of retroviruses (and other viruses as well) for mediating DNA transfer is that the genome of the virus can be deleted of "essential" genes and replaced with the gene(s) of interest (this is now a "defective" retrovirus). Following introduction of these recombinant DNA molecules (as plasmids) into retrovirus "packaging" cell lines (which produce all the proteins required to complete the life

cycle), one can produce retroviral vectors (as infectious retroviruses) in the **absence** of replication- competent virus. Several different retroviral packaging cell lines are available, but only those packaging cell lines with an **amphotropic** host range, that is, producing envelope protein which allows the virus to infect many different cell types across species, produce virus particles that can infect human cells (3). Typical experiments to generate infectious retroviral particles are illustrated below.

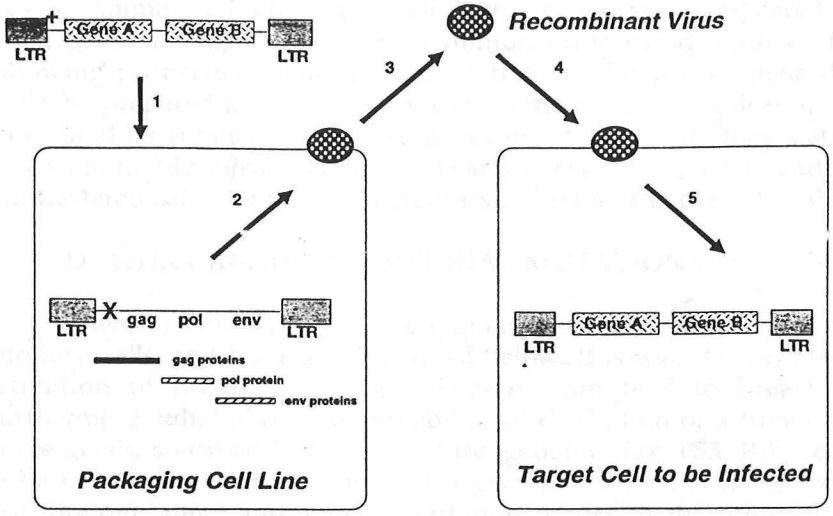


FIG. 2. Schematic representation of recombinant virus production and infection of a target cell. 1. The retroviral vector carrying genes of interest (Genes A and B; for example, a functional copy of the defective gene and a selectable marker gene) are introduced (by gene transfer methods such as transfection, electroporation, *etc.*) into a retroviral packaging cell line. 2. Such a packaging cell line produces the viral structural proteins and enzymes, but is not able to package the viral mRNA encoding these proteins because the ψ region has been deleted (X). These proteins associate with genomic-length RNA from the introduced vector construct, which carries an intact ψ region (+), to form recombinant virus particles. 3. The recombinant virus particles carrying the retroviral vector genome bud out of the packaging cell line into the cell culture medium. 4. The virus-containing medium is either directly filtered to remove cells and cellular debris and then used to infect the target cell, or virus is purified and concentrated before infecting target cells. The infection event is facilitated by the use of agents, such as Polybrene, which are positively charged and help to bring the virus and cell together because they are both negatively charged. 5. After the virus has bound to its cognate receptor on the cell surface, the viral core is delivered into the cell and the viral RNA is reverse-transcribed into a DNA form that integrates into the host cell DNA. The integrated viral DNA (provirus) functions essentially as any other cellular gene and directs the synthesis of the products of Genes A and B. Since the genetic information encoding the viral proteins is not present, no further recombinant virus can be produced by the infected cell.

C. ARE RETROVIRUSES SAFE?

One of the first questions which had to be addressed, assuming retroviruses were to be used for delivery of genes into humans, was whether or not they could be used safely. It was clearly established some of these retroviruses were capable of producing aggressive neoplasms in mice. What about primates? Would they constitute an unacceptable risk to the patient and the personnel administering them? Although all of the clinical protocols approved for use in humans proposed *ex vivo* gene delivery with replication-defective viruses, there was the theoretical possibility that wild-type virus might arise (via recombination of the input DNA with existing retroviral genomes). Therefore, initial studies were conducted in

rhesus monkeys with murine amphotropic retroviruses (4); five monkeys (three normal, two immunosuppressed) were given intravenous infusions (or implants of infected fibroblasts) of high titer viral supernatants (from 12-22% of their blood volume!) and followed for any adverse reactions. No clinical illness resulted in any of the animals (two animals developed asymptomatic lymphadenopathy); in fact, the virus was rapidly cleared from the bloodstream - within 15 minutes of completion of the infusion (via a complement-mediated process). The virus was found to integrate into peripheral blood and lymph node cells and replication-competent retrovirus was found in one of the immunosuppressed animals; at 48.9 mos all animals are clinically well. Thus, retroviruses appeared to be safe, even when given in huge quantities to immunosuppressed animals. Furthermore, the following properties made them attractive candidates for gene delivery: (1) they could be prepared as "defective" viruses - incapable of replicating on their own, (2) they could be engineered to carry genes into cells, where they stably integrated, (3) they were highly infectious, hitting nearly every dividing cell, and (4) they could be administered safely without apparent risk to either the patient or the staff.

D. WHAT ABOUT INSERTIONAL MUTATIONS?

There are basically two consequences of insertion of the retroviral genome into host cells, and these are illustrated below. It is well-documented that insertional activation of dominant-acting oncogenes can lead to breast cancer in mice. Furthermore, what about the possibility of disruption of a tumor suppressor gene? These genes, so-called "guardians of the genome" (ex. P53, RB), require both copies to be mutated in order to provide a growth advantage to the cells - if the virus disrupts one allele and a point mutation occurs in the other, then it is formally possible you might promote tumors as a result of retroviral insertion. After careful follow-up of the monkeys infected with the murine retroviruses, and theoretical calculations of the frequency with which the virus might do something bad (5), the RAC finally decided this was going to be a **very rare** event and approved the viruses for use in humans (initially for marking studies of TILs).

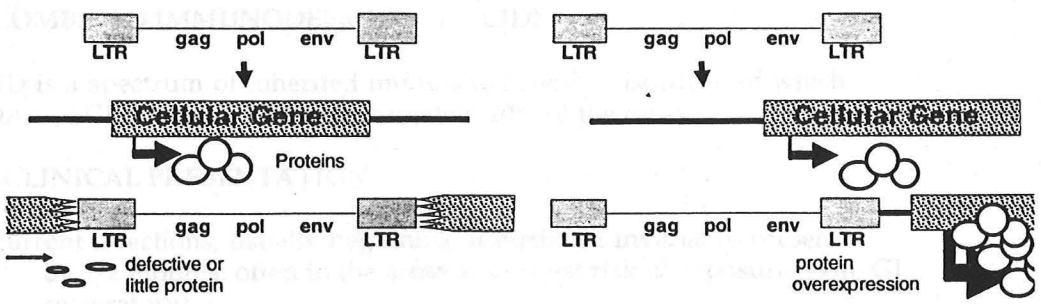


Figure 1. Genetic consequences of insertion of retroviral genomes into host DNA.

III. GENE THERAPY FOR THE IMMUNODEFICIENCIES

The initial enthusiasm for advancing gene therapy into humans focused on the disorders of hemoglobin synthesis. However, by 1984 it had become increasingly clear that the regulation of globin synthesis was very complex (indeed, is not even solved today). It seemed wiser to concentrate efforts on a single gene disorder in which correction of the basic defect would (1) have a significant chance of working, (2) would provide a selective advantage to the corrected cells. Three of the primary immunodeficiency disorders fit the description on theoretical grounds: ADA deficiency, PNP (purine nucleoside phosphorylase) deficiency, and HGPRT deficiency (Lesch-Nyhan syndrome). History seemed to favor ADA deficiency; it was the first disease for which bone marrow transplantation was successfully introduced into clinical practice (6). Indeed, enzyme replacement therapies were also first employed to treat SCID.

Table 1 Subchromosomal locations of the gene loci for the Primary Immunodeficiencies.

Disease	*Location*
Adenosine Deaminase Deficiency	20 q 13.4
Purine Nucleoside Phosphorylase Deficiency	14 q 13.1
Ataxia Telangiectasia	11 q 22–23
X-linked SCID	X q 1.3
Wiskott-Aldrich syndrome	X p 11-11.3
X-linked agammaglobulinemia	X p 21.33-22
X-linked Lymphoproliferative syndrone	X q 26-27
X-linked immunodeficiency with hyper-IgM	X q 26-27
Leukocyte Adhesion Defect (CD18 deficiency)	21 q 22.3
Chronic Granulomatous Disease	
gp91-phox	X p 21.1
p22-phox	16 q 24
p47-phox	7 q 11.23
p67-phox	1 q 25

SEVERE COMBINED IMMUNODEFICIENCY (SCID)

SCID is a spectrum of inherited immunodeficiency disorders, of which ADA (-) SCID accounts for approximately 20% of the cases

A. CLINICAL PRESENTATION

Recurrent infections, usually beginning at birth but invariably present by 1-2 months, often in the areas at greatest risk of exposure (skin, GI, respiratory)
Host of infectious agents (Candidiasis almost invariably)
Delayed physical growth and development
Death due to overwhelming sepsis by age two

B. LABORATORY PRESENTATION

Lymphopenia, moderate to severe (<500/cmm)
Platelet dysfunction
Absence of skin tests to common antigens
Lack of antibody responses to specific antigens (tetanus, blood group)
Lack of lymphocyte markers (when present), absent response to *in vitro* tests
Declining antibody levels as maternal contribution clears
Absence of erythrocyte adenosine deaminase activity, elevated levels of ADA substrates in plasma and urine

C. GENETICS

Rare, < 60 cases world-wide
One third sex-linked, two thirds autosomal recessive (of latter, one third are ADA (-) SCID
Multiple mutations identified in gene (activity, stability, kinetics)

D. PATHOGENESIS

The biochemical basis of the disease is the accumulation of intracellular metabolites (deoxyadenosine, deoxyATP) that are toxic for lymphocytes, particularly T-cells (which have higher levels of the kinase that phosphorylates deoxyAd to dATP), resulting in T-cell lymphopenia (7).

E. TREATMENT

Identical sibling bone marrow, success >90%
HLA-matched bone marrow transplant, success >70%
ADA(-) respond less well than ADA(+)
If absence HLA-match, T-cell depleted parental marrow (with cytoablation)
Attendant complications (GvHD, cytoablation risk, frequent transfusions of supportive products)
Enzyme replacement
PEG-ADA (bovine ADA covalently attached to polyethylene glycol to prolong its half-life); usually given IM weekly
Complications: neutralizing antibodies, persistent infections, absence of sustained correction of T and B cell defects
Cost: $125,000-$250,000 per year
Cytokine therapy
Parenteral IL-2 / PEG-IL-2 (induce clonal expansion of T-cells)

F. GENE THERAPY OF ADA (-) SCID

There is no animal model of ADA deficiency; indeed, prior to 1987 there were no ADA deficient cell lines in which to model replacement strategies*in vitro* . By using HTLV-1 virus, Anderson and colleagues were able to immortalize a series of mature T-cell lines from an ADA deficient patient (JF); one of these T-cell lines displayed properties characteristic of T-cells (CD4+, CD8+, IL-2 receptor + and dependent on IL-2 for growth, and a number of T-cell receptor chain subunit rearrangements). The critical question remained: could you correct the defect by replacing the enzyme in T-cells? Transfer of the human ADA gene into the ADA deficient cell lines via retroviral transduction with the SAX retroviral vector (S - SV40 promoter, A - human ADA gene, X - restriction enzyme cloning site) yielded stable cell lines with normal levels of ADA, and that were 30-100 fold more resistant to the toxic effects of deoxyadenosine than were the original cell lines (8).

Table 1. ADA activity and susceptibility to inhibition by 2'-deoxyadenosine of normal ADA-positive, ADA-deficient, and SAX-transduced T and B cells

Cell type	ADA activity*	IC_{50},† μM
T-cell lines		
HM (normal ADA⁺)	413 ± 55	2940
TJF-2 (ADA⁻)		
Not transduced	4.7 ± 1.5	100
SAX-transduced	147 ± 46	280
SAX-transduced, selected‡	1020 ± 136	2600
SAX-transduced, selected;‡ + 10 μM 2'-deoxycoformycin	<1.0	<100
N2-transduced	4.5	110
B-cell lines		
JM (normal ADA⁺)	454 ± 119	8100
GM2756 (ADA⁻)		
Not transduced	2.7 ± 0.4	210
SAX-transduced	314 ± 99	1100
SAX-transduced, selected‡	1250 ± 134	7400

*The [^{14}C]adenosine assay for ADA activity was performed essentially as described by Van der Weyden and Bailey (24). Results are expressed as nmol of inosine produced per min per 10^8 cells (mean ± SEM for 5 determinations, except N2-transduced TJF-2, for which the value is the result of a single determination).

†Concentration of 2'-deoxyadenosine that inhibits incorporation of [^{3}H]thymidine to 50% of control value after 24 hr of culture.

‡Grown for 3 weeks in medium containing G418 at 1 mg/ml.

Thus, the principle biochemical defect in ADA(-) T-cells could be corrected, at least *in vitro* , via retroviral-mediated gene transfer. A variety of retroviral vectors were then tested for their ability to provide the most efficient gene transfer with stable, high level expression of the human ADA gene (9); the vector LASN (L - 5' LTR with the Moloney murine leukemia virus promoter, A - human ADA gene, S -

SV40 promoter driving the expression of N - neomycin phophotransferase gene to allow for positive selection) provided the highest levels of ADA protein expression. Indeed, the transduced ADA (-) T-cells appeared to have a selective survival advantage and continued to proliferate in cuture (a critical point when the modified clinical protocol went before the RAC).

Two children are currently enrolled in this protocol. Each had been treated with regular PEG-ADA injections for at least two years, with persistent immunodeficiency, prior to initiation of the protocol. The overall protocol for their treatment is shown below.

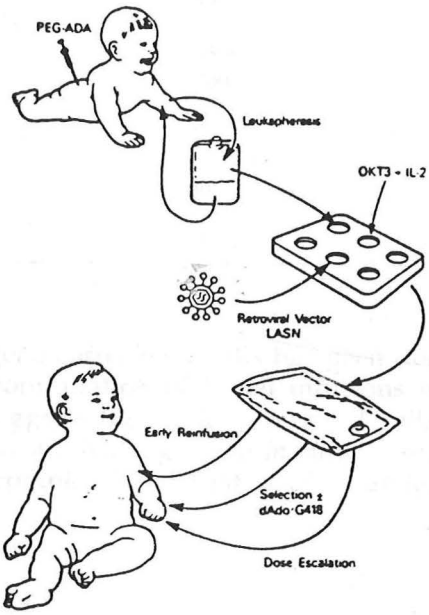


FIG. 1. Schematic of the human ADA gene therapy protocol.

Briefly, the children undergo apheresis to obtain peripheral blood mononuclear cells every 4-12 weeks. These are placed in culture with OKT3 and rIL2 which stimulates vigorous T-cell proliferation; 24 hours later the cells are exposed to LASN supernate twice daily. Approximatley 1-10% of the cells are transduced with the virus. These are then cultured for an additional 9-11 days, during which time the cells have expanded 50-100 fold. They are then infused on the day of treatment; the patient comes in, has an IV started, and twenty minutes later goes home. The results on the first patient are shown on the next series of slides (some data not reproduced here at the request of the investigators).

Table 2 Immune Status of ADA(-)SCID patient#1 before and 10 months after beginning treatment with infusions of ADA gene-corrected autologous T cells. This 4 y/o girl had received regular injections PEG-ADA for 22 months prior to the initiation of lymphocyte gene therapy. Gene therapy treatments consisted of infusions of unselected populations of autologous, culture expanded T cells that had been transduced with the ADA containing retroviral vector LASN. The efficiency of gene transfer ranged from ~1 to 5%. The patient received 7 infusions of gene-corrected T cells during this 10 month period.

Characteristic	*Before Gene Therapy*	*After 10 months*
Total CD3 cells (per μl)	571	1995
PHA response (cpm)	18500	48200
OKT3 response (cpm)	700	29000
Isohemagglutinin titer	1:16	1:64
Tonsils	none	present
Lymphocyte ADA (units)	0.6	8.3

Persistence of the gene-corrected T-cells has been documented in both of the children, despite the discontinuation of T cell infusions in one patient for up to nine months now. This suggests the ADA-corrected T cells may survive for many months and that the survival advantage seen *in vitro* is recapitulated *in vivo* . One of the children has now completed her first year in kindergarten; she missed one day of class.

G. THE FUTURE?

The selective growth advantage for ADA gene-corrected T-cells *in vivo* suggests the genetic correction of totipotent hematopoietic stem cells may also be sufficient to treat the disease (perhaps for life). This would be accomplished by infecting a population of peripheral blood cells enriched for precursor, or stem, cells (eg, CD34+-enriched), or bone marrow cells directly, in hopes the stem cells were stably infected (this has already been done in mice and monkeys (10,11); indeed, one protocol to do this in humans has just been initiated (12).

IV. GENE THERAPY FOR FAMILIAL HYPERCHOLESTEROLEMIA (FH)

FH (receptor negative) is a rare disease, occurring with an estimated frequency of 1:1,000,000 live births. These patients have markedly elevated levels of LDL cholesterol in the serum (often >600 mg%) and are prone to develop accelerated atherosclerosis at an early age. They present clinically with accumulations of LDL-derived cholesterol deposits in multiple sites, primarily tendonous regions (xanthomas) and arteries. These patients are often refractory to pharmacologic therapy, and require aggressive forms of treatment (plasmapheresis, liver transplantation, portacaval shunts) for management.

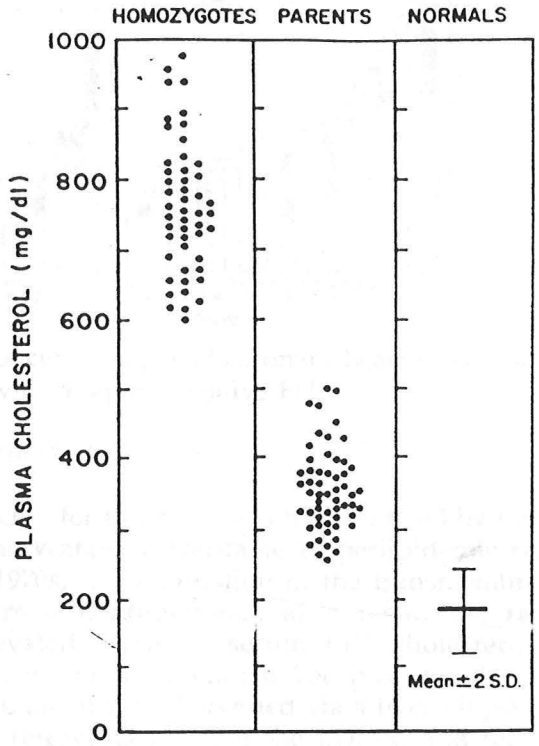


Figure 1. Total plasma cholesterol levels in 49 FH homozygotes, their parents (obligate heterozygotes), and normal controls.

A. GENETICS

The disease is inherited in an autosomal dominant fashion; heterozygotes, numbering 1:500 (in American, European and Japanese populations) have an intermediate serum cholesterol level (350 - 550 mg%) and also develop early complications from atherosclerosis. The frequency of this disorder in the general population makes it one of the most commonly inherited metabolic diseases. We spend approximately $40 billion dollars a year in this country on health care associated in one form or another with complications resulting from atherosclerotic disease.

Receptor negative patients with FH have a dire prognosis as shown in the figure below. The poor prognosis, and the aggressive forms of therapy, make FH (and perhaps hypercholesterolemia in general) an ideal candidate for alternative forms of treatment.

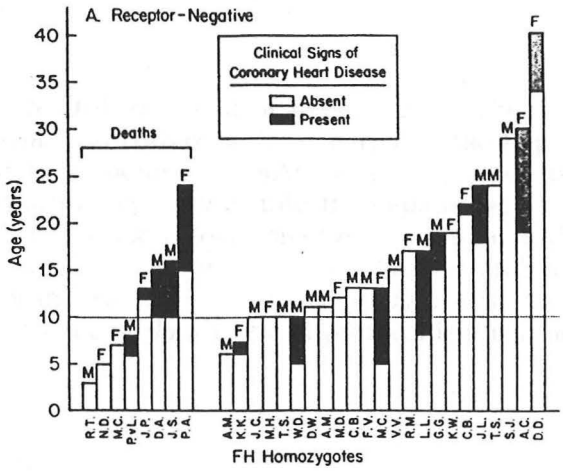


Figure 2. Prevalence of clinical signs of coronary heart disease at different ages in homzygotes with receptor-negative FH.

B. Animal investigations

Gene therapy approaches for this disease were facilitated by the availability of an animal model of FH, the Watanabe Heritable Hyperlipidemic rabbit (WHHL). This animal, described in 1970s, has a mutation in the transmembrane domain of the receptor which renders it nonfunctional; as a result the rabbits develop atherosclerosis due to elevated levels of serum LDL-cholesterol. In order to demonstrate that replacement of a functional receptor would be efficacious, hepatocytes from the WHHL rabbit were harvested via a liver biopsy, established in culture, transduced with a retrovirus carrying the LDL-R, and reinfused into the hepatic vein. Transplantation of the genetically-corrected hepatocytes was associated with a 30-40 % decrease in serum cholesterol, shown below (13).

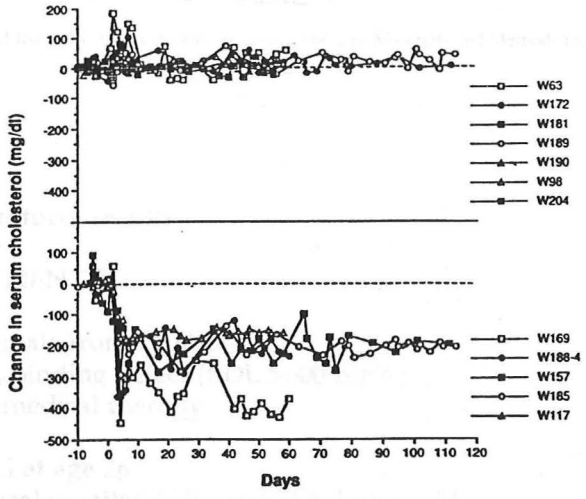


Fig. 3. Effect of hepatocyte transplantation on total serum cholesterol. Baseline amounts of cholesterol were established from determinations made 10 days before transplantation. Assays were performed with the method of Trinder (*17*). Sera were analyzed from each recipient for variable periods of time after transplantation (60 to 121 days). Multiple determinations were made each week. The data are presented as change in serum cholesterol from baseline versus time after transplantation. At the initiation of the study, the animals were 4.0 to 5.0 months old and massed 2.8 to 3.0 kg. **(Top)** WHHL rabbits that received *lacZ*-transduced cells. Baseline serum cholesterol amounts from these animals before treatment were as follows: W63, 534 mg/dl; W172, 685 mg/dl; W181, 501 mg/dl; W189, 565 mg/dl; W190, 478 mg/dl; W98, 671 mg/dl; and W204, 697 mg/dl. **(Bottom)** WHHL rabbits that received LDLR-transduced hepatocytes. Baseline serum cholesterol concentrations from these animals before treatment were as follows: W169, 988 mg/dl; W188-4, 806 mg/dl; W157, 754 mg/dl; W185, 678 mg/dl; and W117, 614 mg/dl.

The successful treatment of hypercholesterolemia in this model suggested it would be possible to correct, at least partially, the hypercholesterolemia present in humans. However, before the RAC would approve the studies in humans, the safety of reinfusing hepatocytes into the hepatic vein had to be assessed. This was accomplished in 3 baboons (over one billion cells infused) without evidence of acute or chronic complications; at two years Wilson and his group could still detect engraftment of the transduced hepatocytes by *in situ* hybridization.

The schematic of the treatment protocol is illustrated below.

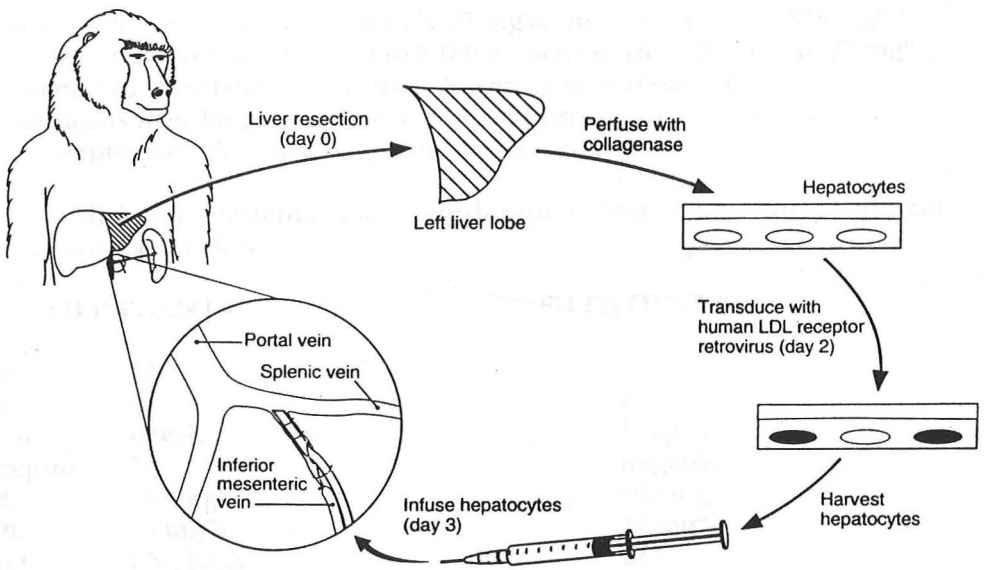


FIG. 2. Schematic diagram of liver resection and catheter placement. See Materials and Methods for description of techniques.

B. Human protocol results

FH PATIENT #1

29 year old female from Quebec
Homozygous, binding defect (LDL >600 mg%)
Refractory to medical therapy
MI at age 16
2-vessel CABG at age 26
Cath 6/92 revealed failed SVG and 75% lesion LM

TREATMENT PROTOCOL (6/92)

Liver resection	250 gm
Collagenase	harvested approximately 3 billion cells (98% viable)
	established as primary cultures in **800** ten cm dishes (!)
	transduced, 48 hours later reinfused 2 billion cells via indwelling catheter in three separate infusions
Complications	tachycardia, principal investigator >> patient

RESPONSE TO HEPATOCYTE INFUSION (at 4 mos)

Cholesterol lowered by approximately 75 mg% (from 600 mg% to 525 mg%)
LDL/HDL ratio lowered from 12 to 7 (HDL increase from 50 mg% to 75 mg%)
Responded to lovastatin with further lowering of cholesterol
Percutaneous liver biopsy (at 4 mos) revealed presence of transduced heaptocytes (detected by specific *in situ* probe)

Two additional patients have undergone treatment; their clinical characteristics are listed below.

	FH PATIENT #2	FH PATIENT #3 *
Age	**12**	**7**
Sex	**M**	**F**
Ethnicity	**Greek**	**Hispanic**
Receptor	**?**	**negative**
LDL	**550 mg%**	**900 mg%**
HDL	**35 mg%**	**20 mg%**
CAD	**LM, RCA**	**LM**

*This patient's sister died at age 3 of a myocardial infarction.

These studies have demonstrated the feasibility of transducing primary cultures of hepatocytes with functional LDL-R that result in a lowering of cholesterol (albeit a rather "unimpressive" lowering). However, considering that far less than 1% of the hepatocytes following reinfusion and engraftment demonstrate a positive *in situ* reaction, this might not be so unimpressive. If techniques could be improved, for example, 100% of the hepatocytes transduced in culture (with each cell producing 10 X the normal level of the LDL-R), a more dramatic and sustained lowering of cholesterol might be achieved (assuming there are 250 billion cells in an adult liver, the genetic reconstitution would approach 10%).

C. THE FUTURE?

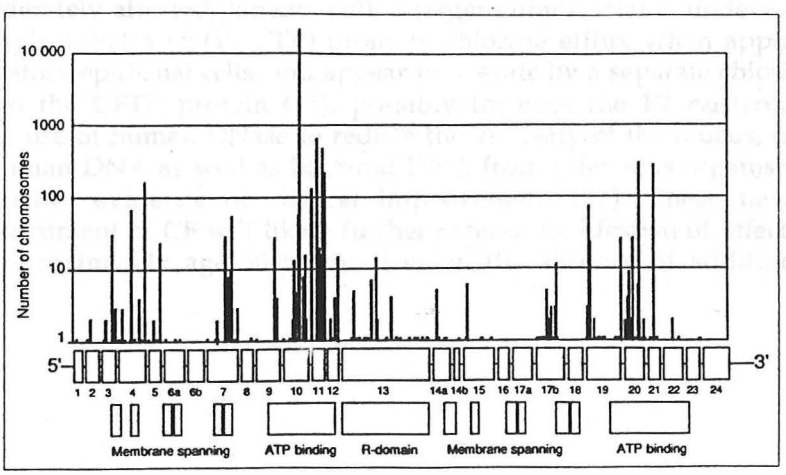
Alternatives to *ex vivo* gene therapy are currently being developed. Drs. Herz and Gerard at this institution have demonstrated that an infusion of adenovirus,

carrying a functional LDL-R, into the tail vein of LDL-R deficient mice (generated by gene-targeting in ES cells) lowers the serum cholesterol (14). The cholesterol-lowering effect does not persist and the animals are resistant to a second infusion of virus (probably due to neutralizing antibodies). Nonetheless, methods which would allow persistent expression of the therapeutic gene would greatly facilitate the treatment of hypercholesterolemia in humans. Is it possible to target the therapeutic gene, either by modifying the delivery vector or the controlling elements? These are areas of intense investigation in numerous laboratories around the world, and in my view have the greatest potential in terms of research careers for physician (and non-physician) scientists.

V. GENE THERAPY FOR CYSTIC FIBROSIS

Cystic fibrosis (CF) is a complex inherited disorder affecting primarily children and young adults. It is inherited in an autosomal recessive fashion, and heterozygotes are entirely asymptomatic. The frequency of the disease varies among ethnic groups and is highest in individuals of Northern European extraction where the frequency of the mutation is 1 in 25 individuals. It was originally described by Anderson in 1938, and in 1953 DiSant'Agnese demonstrated that excessive salt loss occurred in the sweat of children with the disease . The sweat chloride test remained the standard test for the disease for many years, but the basic defect remained a mystery. In 1983 Quinton demonstrated an attenuated chloride transport in sweat ducts (15), followed soon thereafter by demonstration of a similar defect in the respiratory epithelium (16).

Perhaps one of the most significant advances in CF diagnosis occurred with the identification of the gene defect in 1989 by several different groups working together (17-19). The gene is over 250,000 bp, encoding a transcript of about 6.5 kb and a protein of 1480 amino acids. The protein product is known as CFTR for cystic fibrosis transmembrane conductance regulator. A three bp deletion in exon 10 results in the in frame loss of a single amino acid, designated delta F508 for the loss of phenylalanine, and accounts for approximately 70% of the mutations. However, over 230 different mutations have subsequently been identified (at present we can identify 85-90% of carriers, and the potential for population screening is under way in several pilot projects in the US, Canada and Europe).



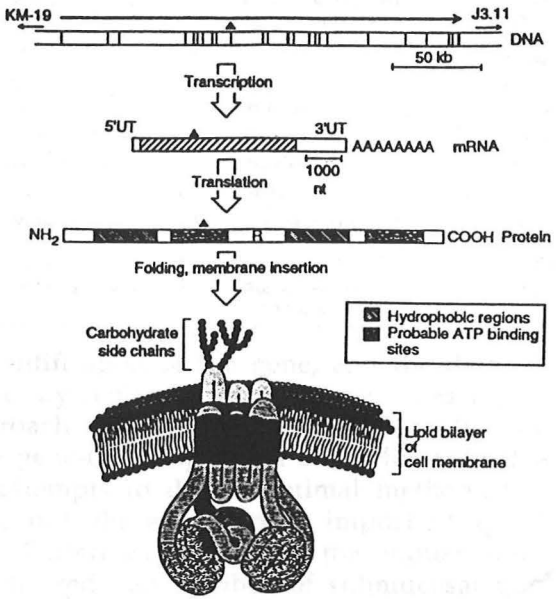


Figure 1. Gene, transcript and predicted structure for the CFTR protein. The solid triangle shows the site of the delta F508 mutation.

It is now clearly established that the CFTR protein is a chloride channel, and that introduction of the normal CFTR protein into cells lacking the normal gene confers chloride transport that is activated by cAMP.

The Table on the following page lists the current approaches to treatment of CF. Three new clinical trials are in progress. In addition to the well-known chloride defect, there is also an increase in sodium uptake by the respiratory epithelium, resulting in a dehydration of the airway mucus by enhancing salt and water uptake. Amiloride, a potassium-sparing diuretic, blocks the uptake of sodium ions by respiratory epithelium and may prevent the mucus from beconing so thick and viscous. In one clinical trial, aerosolized amiloride slowed the pulmonary deterioration of moderately affected patients (20); a larger clinical trial is underway. Likewise, certain triphosphates (ATP, UTP) promote chloride efflux when applied directly to the respiratory epithelial cells, and appear to operate by a separate chloride efflux pathway than the CFTR protein (21), possibly through the P2 nucleotide receptor. Finally, the use of human DNase to reduce the viscosity of the mucus, due at least in part ot human DNA as well as bacterial DNA from infectious organisms, has resulted in further evidence of clinical improvement (22). These newer approaches for the treatment of CF will likely further extend the lifespan of affected individuals from approximately age 30 today, even in the absence of additional therapies.

Table 1. Approaches to CF lung disease.

Abnormality	Solution	Approach
Abnormal CF gene	Provide normal gene	Gene therapy
Abnormal CFTR protein	Provide normal protein Activate mutant form	Protein therapy ? Phosphodiesterase inhibitors ? Phosphatase inhibitors ? Others
Abnormal salt transport	Block Na^+ uptake Increase Cl^- efflux	Amiloride ATP/UTP
? Abnormal mucus	Decrease viscosity	DNase
Impaired clearance	Augment ciliary action	Chest percussion
? *Pseudomonas* infection	Reduce bacterial count	Antibiotics
Inflammatory response	Decrease host reaction	Antiproteases Anti-inflammatory drugs (steroids, ibuprofen)
Bronchiectasis	Replace irreversibly damaged areas	Lung transplantation

The identification of the gene, and the demonstration of correction of the chloride defect by replacement of the gene, has raised the hopes of using a gene therapy approach to treat the disease. Recently, an animal model of CF was generated by gene-targeting in ES cells; this animal will likely prove to be very valuable in attempts to define optimal methods for introducing the gene into airway cells, but there are some important questions which are currently unanswered. Differences between the mouse and human airway anatomy, particularly the reduced number of submucosal glands in the mouse, make it impossible to predict how useful the mouse will be. Furthermore, most of the mice die of intestinal obstruction before they actually develop lung pathology (the intestinal phenotype in the mouse is more severe than the human phenotype).

Adenoviral vectors have been shown to accurately deliver the CFTR gene into airway epithelial cells in the cotton rat (23). This adenoviral vector has subsequently been approved for use in humans.

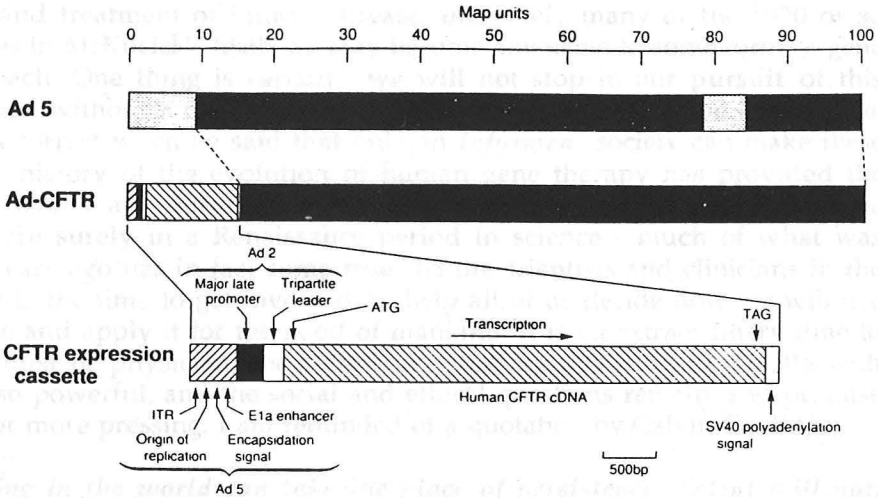


Figure 1. Schematic of the Recombinant Adenoviral Vector Ad-CFTR

Despite these exciting scientific advances, important questions remain and will need to be answered. These are the subject of both animal investigations as well as human trials.

(1) **What are the relevant cells to treat, and when?**
(2) **What fraction of the affected cells must be corrected before clinical benefit can be expected?**
(3) **How will the gene be delivered and its expression controlled?**
(4) **How long will expression persist, and can you retreat without complications?**
(5) **Can we insure it will be safe, and not infect other cell types (eg germ cells)?**

There are no clear answers to these questions, although several clinical protocols have been approved by the RAC for human applications. One trial, under the direction of Dr. Jeff Whitsett at the University of Cincinnatti in collaboration with Genetic Therapy, Inc. will use adenovirus vectors carrying a functional copy of the CFTR in a Phase I trial this month. A second Phase I trial is underway at the University of North Carolina to assess the safety of infecting nasal airway cells with an E!-deleted adenovirus carrying the CFTR gene. The preliminary results of these trials will probably be reported at upcoming human gene therapy meetings.

VI. THE FUTURE

Over the past twenty five years we have witnessed extraordinary advances in our knowledge of the basic biochemical and genetic defects in human disease. With the large scale sequencing of the human genome, and the rapid pace at which disease genes have been identified only within the past two years, it is predicted the pace will only increase. Indeed, much of the next century of science will probably be dedicated to figuring out **how** most of these genes actually function. With each new advance in our understanding of these defects will come new insights into pathogenesis and treatment of human disease; ultimately many of the 4000 or so genetic diseases in McKusick's textbook may become amenable to some form of gene therapy approach. One thing is certain - we will not stop in our **pursuit** of this knowledge, even without a clear sense of what will be **done with** the knowledge. Anderson was correct when he said that only an ***informed*** society can make these decisions. The history of the evolution of human gene therapy has provided the paradigm by which any future technological advances in humans should be pursued. We are surely in a Renaissance period in science - much of what was predicted 25 years ago has in fact come true. To the scientists and clinicians in the audience, now is the time to get involved, to help all of us decide how we will use this knowledge and apply it for the good of mankind. It is an extraordinary time to become a scientist or physician; the techniques that are available to wrestle with problems are so powerful, and the social and ethical questions remain, and promise to become, ever more pressing. I am reminded of a quotation by Calvin Coolidge,

" Nothing in the world can take the place of persistence. Talent will not; nothing is more common than unsuccessful men with talent. Genius will not; unrewarded genius is almost a proverb. Education will not; the world is full of educated derelicts. Persistence and determination alone are omnipotent. The slogan "Press on" has solved, and always will solve, the problems of the human race. "

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(21) Knowles MR, et. al. Activation by extracellular nucleotides of chloride secretion in the airway epithelium of patients with cystic fibrosis. *NEJM* 325:533-538 (1991).
(22) Hubbard, RC, et. al. A preliminary study of aerosolized recombinant human deoxyribonuclease I in the treatment of cystic fibrosis. *NEJM* 326:812-815 (1992).

RECOMMENDED READING

I recommend the entire first issue of Human Gene Therapy (Spring 1990); there are several articles dealing with ethics/legal/regulatory issues that are highly informative.

The **Molecular Advances** section in the 8 May 1992 issue of Science; there are nine articles dealing with subjects as diverse as biotech companies to Alzheimer's.

The **Frontiers in Biotechnology** section in the 14 May 1993 issue of Science; seven articles including one of my favorites by RC Mulligan entitled "The Basic Science of Gene Therapy".

To obtain copies and updates on current clinical protocols you can contact the RAC directly; ask to speak to Dr. Nelson Wivel, he is extremely knowledgable and helpful about where to find information. The list below are the current protocols approved by the RAC and the NIH (where indicated).

RAC (301) 496-9838 Phone
(301) 496-9839 FAX

HUMAN GENE TRANSFER (TR) / THERAPY(TH) PROTOCOLS

8810-001(TR) Rosenberg, Steven A., National Cancer Institute; *The Treatment of Patients with Advanced Cancer Using Cyclophosphamide, Interleukin-2 and Tumor Infiltrating Lymphocytes.*
Date of RAC Approval: 10/3/33
Date of NIH Approval: 3/2/89

9007-002** (TH) Blaese, R., Michael, National Cancer Institute; *Treatment of Severe Combined Immune Deficiency (SCID) due to Adenosine Deaminase (ADA) Deficiency with Autologous Lymphocytes Transduced with a Human ADA Gene.*
Date of RAC Approval: 7/3/1/90
Date of NIH Approval: 9/6/90

9007-003(TH) Rosenberg, Steven A., National Cancer Institute; *Gene Therapy of Patients with Advanced Cancer Using Tumor Infiltrating Lymphocytes Transduced with the Gene Coding for Tumor Necrosis Factor.*
Date of RAC Approval: 7/31/90
Date of NIH Approval: 9/6/90

9102-004(TR) Brenner, Malcolm K.; Mirro, Joseph; Hurwitz, Craig; Santana, Victor; and Ihle, James, St. Jude Children's Research Hospital; *Autologous Bone Marrow Transplant for Children with Acute Myelogenous Leukemia in First complete Remission: Use of Marker Genes to Investigate the Biology of Marrow Reconstitution and the Mechanism of Relapse.*
Date of RAC Approval: 5/3/1/91
Date of NIH Approval: 7/12/91

9105-005(TR) Brenner, Malcolm K.; Mirro Joseph; Santana, Victor; and Ihle, James, St. Jude Children's Research Hospital; *A Phase I/II Trial of High Dose Carboplatin and Etoposide with Autologous Marrow Support for Treatment of Stade D Neuroblastoma in First Remission: Use of Marker Genes to Investigate the Biology of Marrow Reconstitution and the Mechanism of Relapse.*
Date of RAC Approval: 5/31/91
Date of NIH Approval: 7/12/91

9105-006(TR) Brenner, Malcolm K; Mirro, Joseph; Santana, Victor; and Ihle, James, St. Jude Children's Research Hospital; *A phase II Trial of High-dose Carboplatin and Etoposide with Autologous Marrow Support for Treatment of Relapse/Refractory Neuroblastoma Without Apparent Bone Marrow Involvement.*
Date of RAC Approval: 5/31/91
Date of NIH Approval: 7/12/91

9105-007(TR) Deisseroth, Albert B., M.D. Anderson Cancer Center; *Autologous Bone Marrow Transplantation for Chronic Myelogenous Leukemia in which Retroviral Markers are Used to Discriminate between Relapse which Arises from Systemic Disease Remaining after Preparative Therapy Versus Relapse due to Residual Leukemic Cells in Autologous Marrow: A Pilot Trial.*
Date of RAC Approval: 5/31/91
Date of NIH Approval: 7/12/91

9105-008(TR) Ledley, Fred D.; Woo, Savio; Ferry, George; and Hartweel Whigennand, Baylor College of Medicine; *Hepatocellular Transplantation in Acute Hepatic Failure and Targeting Genetic Markers to Hepatic Cells.*
Date of RAC Approval: 5/30/91
Date of NIH Approval: 7/12/91

9105-009*(TR) Lotze, Michael T., University of Pittsburgh School of Medicine; *The Administration of Interleukin-2, Interleukin-4, and Tumor Infiltrating Lymphocytes to Patients with Melanoma.*
Date of RAC Approval: 5/30/91
Date of NIH Approval: 1/17/92
Minor Modification: 11/30/92

9110-010*(TH) Rosenberg, Steven A., National Cancer Institute; *Immunization of Cancer Patients Using Autologous Cancer Cells Modified by Insertion of the Gene for Tumor Necrosis Factor (TNF).*
Date of RAC Approval: 10/7/91
Date of NIH Approval: 10/15/91
Minor Modification: 7/9/92

9110-011(TH) Rosenberg, Steven A. National Cancer Institute; *Immunization of Cancer Patients Using Autologous Cancer Cells Modified by Insertion of the Gene for Interleukin-2 (IL-2)*
Date of RAC Approval: 10/7/91
Date of NIH Approval: 10/15/91

9110-012*(TH) Wilson, James M., University of Michigan; *Ex Vivo Gene Therapy of Familial Hypercholestorolemia.*
Date of RAC Approval: 10/8/91
Date of NIH Approval: 11/14/91
Minor Modification: 12/3/92

9202-013*(TH) Nabel, Gary J., University of Michigan; *Immunotherapy of Malignancy by In Vivo Gene Transfer into Tumors.*
Date of RAC Approval: 2/10/92
Date of NIH Approval: 4/17/92
Minor Modification: 1/22/93

9202-014(TR) Cornetta, Kenneth, Indiana University;-*RetroviralMediated Gene Transfer of Bone Marrow Cells during Autologous Bone Marrow Transplantation for Acute Leukemia.*
Date of RAC Approval: 2/11/92
Date of NIH Approval: 4/17/92

9202-015(TR) Economous, James S. and Belledegrun, Arie, University of California at Los Angeles; *The Treatment of Patients with Metastatic Melanoma and Renal Cell Cancer Using In Vitro Expanded and Genetically Engineered (Neomycin Phosphotranferase) Bulk, CD8 (+) and/or CD4 (+) Tumor Infiltrating Lymphocytes and Bulk, CD8 (+) and/or CD4 (+) Peripheral Blood Leukocytes in Combination with Recombinant Interleukin-2. Alone, or with Recombinant Interleukin-2 and Recombinant Alpha Interferon.*
Date of RAC Approval: 2/11/92
Date of NIH Approval: 4/17/92

9202-016(TH) Freeman, Scott M., University of Rochester School of Medicine, Rochester, New York; *Gene Transfer for the Treatment of Cancer.*
Date of RAC Approval: 2/10/92
Date of NIH Approval: 2/5/93

9202-017(TR) Greenberg, Philip D. and Riddell, Stanley, University of Washington, Seattle; *Phase I Study of Cellular Adoptive immunotherapy Using Genetically Modified CD8+HIV-Specific T Cells for HIV-Seropositive Patients Undergoing Allogeneic Bone Marrow Transplant.*
Date of RAC Approval: 2/11/92
Date of NIH Approval: 4/17/92

9206-018(TH) Brenner, Malcolm K.; Furman, Wayne; Santana, Victor; Bowman, Laura; and Meyer, William, St. Jude Children's Research Hospital; *Phase I Study of Cytokine-Gene Modified Autologous Neuroblastoma Cells for Treatment of Relapsed/Refractory Neuroblastoma.*
Date of RAC Approval: 6/1/92
Date e of NIH Approval: 8/14/92

9206-019(TH) Oldfield, Edward, National Institutes of Health; Gene *Therapy for the Treatment of Brain Tumors Using Intra-Tumoral Transduction with the Thymidine Kinase Gene and Intravenous Ganciclovir.*
Date of RAC Approval: 6/1/92
Date of NIH approval 8/14/92

9206-020(TR) Deisseroth, Albert B., MD Anderson Cancer Center; *Use of two Retroviral Markers to Test Relative Contribution of Marrow and Peripheral Blood Autotogous Cells to Recovery After Preparative Therapy.*
Date of RAC Approval: 6/2/92
Date of NIH Approval: 8/14/92

9206-021(TH) Gansbacher, Bernd; Houghton, Alan; and Livingston, Philip, Memorial Sloan Kettering Cancer Center; *Immunization with HLA-A2 matched Allogeneic Melanoma Cells that Secrete Interleukin-2 in Patients with Metastatic Melanoma.*
Date of Approval: 6/2/92
Date of NIH Approval: 8/14/92

9206-023(TR) Gansbacher, Bernd; Motzer, Robert; Houghton, Alan; and Bander, Neil, Memorial Sloan Kettering Cancer Center; *Immunization with Interluekin-2 Secreting Allogeneic HLA-A2 Matched Renal Cell Carcinoma Cells in Patients with Advanced Renal Cell Carcinoma.*
Date of RAC Approval: 6/2/92
Date of NIH Approval: 8/14/92
Minor Modification: 3/31/93

9206-023 (TR) Dunbar, Cynthia, National Institutes of Health; *Retroviral-Mediated Gene Transfer of Bone Marrow and Peripheral Blood Stem Cells During Autologous Bone Marrow Transplantation for Multiple Myeloma.*
Date of RAC Approval: 6/2/92
Date of NIH Approval: 8/14/92

9206-024(TR) Dunbar, Cynthia, National Institutes of Health; *Retroviral-Mediated Gene Transfer of Bone Marrow and Peripheral Blood Stem Cells During Autologous Bone Marrow Transplantation for Metastatic Breast Cancer.*
Date of RAC Approval: 6/2/92
Date of NIH Approval: 8/14/92

9206-025(TR) Dunbar, Cynthia, National Institutes of Health; *Retroviral-Mediated Gene Transfer of Bone Marrow and Peripheral Blood Stem Cells During Autologous Bone Marrow Transplantation for Chronic Myelogenous Leukemia.*
Date of RAC Approval: 6/2/92
Date of NIH Approval: 8/14/92

9209-026(TR) Walker, Robert E., National Institute of Health; *A study of the Safety and Survival of the Adoptive Transfer of Genetically Marked Syngeneic Lymphocytes in HIV Infected Identical Twins.*
Date of RAC Approval: 9/14/92

9209-027(TR) Schuening, Friedrich G., Fred Hutchinson Cancer Research Center; *Phase I/II study of the Use of Recombinant Human Interleukin 3 (rhil3) Stimulated Peripheral Blood Progenitor Cell Supplementation in Autologous Bone Marrow Transplantation in Patients with Breast Carcinoma or Hodgkins's Disease.*
Date of RAC Approval: 9/14/92
Date of NIH Approval: 2/5/93

9209-028(TR) Schuening, Friedrich G., Fred Hutchinson Cancer Research Center; *Evaluation of the Use of Recombinant Human G-CSF Stimulated Peripheral Blood Progenitor Cell Supplementation in Autologous Bone Marrow Transplantation in Patients with Lymphoid Malignancies.*
Date of RAC Approval: 9/14/92
Date of NIH Approval: 2/5/93

9209-029(TR) Schuening, Friedrich G., Fred Hutchinson Cancer Research Center; *A Trial of G-CSF Stimulated Peripheral Blood Stern Cells for Engraftment in Identical Twins.*
Date of RAC Approval: 9/14/92
Date of NIH Approval: 2/5/93

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