Eukaryotic Gene Expression: Basics & Benefits

P N RANGARAJAN

Lecture 33

Human Gene Therapy

Eukaryotic protein expression systems-II (lecture 31)

Protein expression in mammalian cells (non viral vectors)

~~Cell-free protein expression systems~~

Eukaryotic protein expression systems-III (lecture 32)

Protein expression in mammalian cells (viral vectors)

Human gene therapy (lecture 33)

Can the non viral and viral vectors be directly introduced into the tissues of animals including humans to cure a disease?

YES

Gene Therapy

Is the treatment of genetic or acquired disorders by delivering normal genes to correct the disease.

Gene therapy is a technique used for correcting defective genes that are responsible for disease development.

There are many other approaches to correct a defective gene:

1. A normal gene is expressed (supplementation)
2. A n abnormal gene exchanged for a normal gene (replacement)
3. Suppress the expression of the defective gene (suppression)
4. An abnormal gene repaired through selective reverse mutation (repair)

PROTEIN THERAPY *VS* GENE THERAPY

In protein therapy, the desired gene is expressed in *E. coli*, yeast, insect or mammalian cells and the purified protein is provided to the humans.

Type I Diabetes	Insulin
Pitutary dwarfism	growth hormone
Haemophilia	factor VIII /IX
Anaemia	Erythropoietin

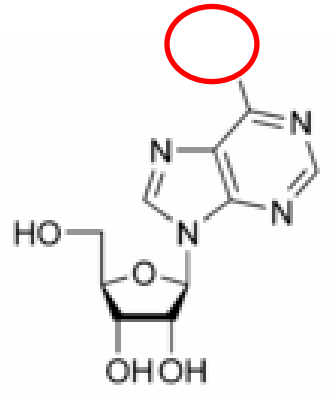
In gene therapy, the desired gene is directly expressed in the cells/tissues of humans

The first human gene therapy was performed on September 14, 1990.

Ashanti DeSilva (four year old girl) was treated for

SCID - Severe combined immunodeficiency)

Ashanti DeSilva was born with a crippled immune system, because of a genetic defect in her genes encoding an enzyme known as Adenosine deaminase (ADA). ADA irreversibly deaminates adenosine to inosine.



In the absence of ADA, toxic adenosine and deoxyadenosine, accumulate in the cells of patients. Lymphoid cells, especially T cells are particularly sensitive to these nucleotides.

Without T cells, ADA-deficient children are wide open to the attacks of viruses and bacteria. These children have what's called severe combined immune deficiency (SCID) disorder, more commonly known as bubble boy disease.

SCID patients

> 8 new ear infections per year

> 2 serious sinus infections per year

> 2 pneumonias per year

> 2 month on antibiotics with little effect

lymphopenia (absolute lymphocyte count less than 200)

-- failure to gain weight and grow

-- recurrent deep skin and organ abscesses

The standard treatment for ADA deficiency is frequent injections of PEG-ADA, a synthetic form of the ADA enzyme.

PEG-ADA can mean the difference between life and death for an ADA-deficient child.

Unfortunately, although it usually produces a rapid improvement when first used, children tend to respond less and less to the drug each time they receive a dose.

Ashanti DeSilva started receiving PEG-ADA injections at the age of two, and initially she responded well.

Her T-cell count rose sharply and she developed some resistance to disease.

But by the age of four, she was slipping away, no longer responding strongly to her injections.

If she was to live, she would need something more than PEG-ADA.

The only other option at the time, a bone-marrow transplant, was ruled out by the lack of matching donors.

To a person with a weak immune system, the outside world is threatening.

Everyone you touch, share a glass with, or share the same air with is a potential source of dangerous pathogens.

Lacking the ability to defend herself, Ashanti was largely confined to her home.

In early 1990, while Ashanti's parents were searching frantically for help, **French Anderson,** a geneticist at the National Institutes of Health, was seeking permission to perform the first gene-therapy trials on humans.

Anderson was trying to get permission to treat patients suffering from genetic defects by introducing normal genes.

Starting in June of 1988, Anderson's proposed clinical protocols, or treatment plans, went through intense scrutiny and generated more than a little hostility.

His first protocol was reviewed by both the National Institutes of Health (NIH) and the Food and Drug Administration (FDA) in the United States.

Over a period of seven months, seven regulatory committees conducted fifteen meetings and twenty hours of public hearings to assess the proposal.

In early 1990, Anderson and his collaborators received the final approval from the NIH's Recombinant DNA Advisory Committee and had cleared all legal hurdles.

By spring, they had identified Ashanti as a potential patient.

Would her parents consent to an experimental treatment?

Of course there were risks to the therapy, yet without it Ashanti would face a life of seclusion and probably death in the next few years.

Given these odds, her parents opted to try the therapy.

Ashanti and her parents flew to the NIH Clinical Center at Bethesda, Maryland.

There, over a period of twelve days, Anderson and his colleagues Michael Blaese and Kenneth Culver isolated Ashanti's T cells and transfected them with a retrovirus expressing the ADA gene and these genetically modified cells were reintroduced back into her body on the afternoon of **September 14, 1990.**

What precisely was done to Ashanti DeSilva?

Her T cells were:
-- placed in tissue culture

-- stimulated to proliferate
(by treating them with the IL-2)

-- infected with a retroviral vector
MoMLV-ADA

-- returned to her in a series of treatments

The injections had to be repeated
because T cells live
for only 6-12 months in the blood

The impact of the gene therapy on Ashanti was striking.

Within six months, her T-cell count rose to normal levels.

Over the next two years, her health continued to improve, allowing her to enroll in school, venture out of the house, and lead a fairly normal childhood

1990

2003

After Ashanti's treatment, the field of gene therapy blossomed.

Since 1990, hundreds of labs have begun experimenting with gene therapy as a technique to cure disease, and more than five hundred human trials involving over four thousand patients have been launched.

Researchers have shown that it may be possible to use gene therapy to cure diabetes, sickle-cell anemia, several kinds of cancer, Huntington's disease and even to open blocked arteries.

Strategy:

Once the therapeutic gene is known, it is cloned into viral or nonviral vectors and introduced into the body

The type of gene therapy performed on Ashanti De Silva is known as :

***Ex vivo* gene therapy -**

Cells are altered outside of patient's body and returned.

***In situ* gene therapy -**

Healthy gene and vector DNA are injected at isolated site on body that is easily accessible (e.g. skin tumor).

***In vivo* gene therapy -**

Vector and gene are directly introduced into the body.

Germline *versus* Somatic Gene Therapy

Somatic gene therapy

- Involves alteration of the DNA of somatic cells implicated in the disease.
- Changes are not heritable.

Germline gene therapy

- Involves alteration of the DNA of a gamete or fertilized egg.
- Changes are heritable; passed from treated individual to offspring.
- Currently, there is no germline gene therapy done in humans.

Genetic diseases: monogenic vs polygenic

Type 1:
Single locus (gene) is defective and responsible for the disease, 100% heritable.

examples:
- Sickle cell anemia,
- Hypercholesterolemia
- Cystic fibrosis
- Haemophilia
- SCID

Type 2:
Polygenic traits, <100% heritable, may be dependent on environmental factors and lifestyle.

examples:
- Heart disease
- Cancer
- Diabetes
- Alcoholism
- Schizophrenia

Inherited/monogenic disorders:

ADA deficiency
Alpha-1 antitrypsin
Chronic granulomatous disease
Cystic fibrosis
Familial hypercholesterolemia
Fanconi Anemia
Gaucher Disease
Hunter syndrome
Parkinsons
Hemophilia

Examples of human gene therapy for genetic disorders

Haemophilia

Hemophilia is an X-linked disorder affecting 1 in 10,000 males

The disease is due to a defect in gene encoding the blood Clotting factor, FACTOR VIII

The normal circulating levels of Factor VIII is 200 ng/ml (1 unit)

Maintainence of of 0.1-0.2 units is accepted as clinically significant

Introduce the gene encoding factor VIII into patients suffering from hemophilia using viral or non viral vectors such that clinically significant levels of factor VIII can be produced in their bodies

Familial hypercholesterolaemia

Familial hypercholesterolemia is a genetic disorder characterized by high cholesterol levels, specifically very high levels of low-density lipoprotein (LDL, "bad cholesterol"), in the blood and early cardiovascular disease.

Many patients have mutations in the *LDLR* gene that encodes the LDL receptor protein, which normally removes LDL from the circulation, or apolipoprotein B (ApoB), which is the part of LDL that binds with the receptor.

Perform *ex vivo or in vivo* gene therapy using viral or nonviral vectors encoding the LDL receptor gene

Ornithine transcarbamylase deficiency

- Deficiency of ornithine transcarbamylase (OTC) is inherited as an X-linked recessive mutation.
- OTC normally breaks down amino acids present in dietary protein.
- Lack of OTC allows build up of ammonia which damages brain function.
- Low protein diets and ammonia-binding drugs are used to treat OTC deficiency.
- Clinical trials to treat OTC deficiency were established using adenovirus as a vector for the normal OTC gene.
- Adenovirus has been used in over 330 gene therapy trials in 4,000 patients.

Examples of human gene therapy for acquired disorders

GENE THERAPY FOR THE TREATMENT OF CARDIOVASCULAR DISORDERS

Revascularization of Blocked coronary arteries leading to myocardial infarction

Gene therapy aims at introducing vectors encoding angiogenic factors (VEGF, FGF) that promote the growth of new arteries leading to revascularization of blocked arteries at the site of ischaemia.

Local expression of anti-thrombotic genes

Gene therapy aims at dissolving blood clots following angioplasty by introducing vectors encoding cyclooxigenase-I (COX-I) involved in the synthesis of Prostacylin (PGI_2), an anti-thrombotic agent.

Gene therapy for the treatment of cancer

Treatment of metastatic melanoma by adoptive immunotherapy using tumour infiltrating lymphocytes (TILs) and with vectors encoding interleukin-2

In vivo / ex vivo gene therapy using viral or non viral vectors encoding tumour suppressor genes, cytokines, alloantigens etc.

Gene therapy for arthritis

Delivering antiarthritic genes intraarticularly to the individual joint, where their expression leads to the accumulation of sustained, therapeutic levels of the gene product.

Human clinical trials of arthritis gene therapy
(Rheumatoid arthritis and osteoarthritis)

Transgene	**Vector, *ex vivo/in vivo***
IL-1 receptor antagonist	Retrovirus, *ex vivo*
IL-1 receptor antagonist	Retrovirus, *ex vivo*
HSV-tk	Plasmid, *in vivo*
TNFR:Fc fusion protein (etanercept)	AAV, *in vivo*
$TGF\beta_1$	Retrovirus, *ex vivo*
$TGF\beta_1$	Retrovirus, *ex vivo*
TNFR:Fc fusion protein (etanercept)	AAV, *in vivo*

3 major challenges in gene therapy

delivery

sustained expression

regulation

1) **Package the gene**
2) **Protect the gene**
3) **deliver the gene to the nucleus and express**

Safe and efficient vectors

Ideal vector for gene theapy

Insert size:	one or more genes.
Targeted:	limited to a cell type.
Immune response:	none.
Stable:	not mutated.
Production:	easy to produce high concentrations or titer.
Regulatable:	produce enough protein to make a difference.

Human gene therapy trials

Vector	Number of trials	Percentage of total trials
Adenovirus	331	24.7
Retrovirus	305	22.8
Vaccinia	91	6.8
Poxviruses	86	6.4
Adeno-associated virus	47	3.5
Herpes simplex virus	42	3.2
Nonviral	343	25.6
Other	93	7.0
Total	1,338	100

Source: Gene therapy clinical trials worldwide to 2007 – an update.
J Gene Med 2007, 9:833-842.

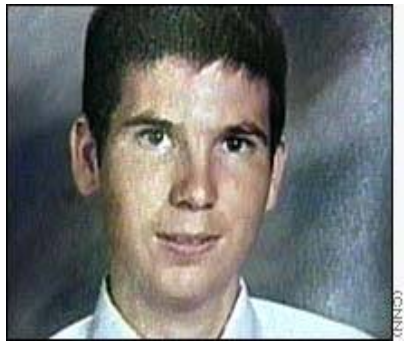
PROBLEMS
&
FAILURES

Human gene therapy failures leading to death of patients

Year	Disease target	Vector
1999	Ornithine transcarbamylase deficiency	Adenovirus
2002	X-linked severe combined immunodeficiency	Retrovirus
2006	X-linked chronic granulomatous disease	Retrovirus
2007	Rheumatoid arthritis	Adeno-associated virus

Gene therapy of Jesse Gelsinger

Ornithine transcarbamylase (OTC) deficiency



Case study: Jesse Gelsinger (18 year old)

Gene therapy began Sept. 13, 1999,
Coma on Sept. 14,
Brain dead and life support terminated on Sept. 17, 1999
Cause of death: Respiratory Disease Syndrome

Conclusions form J.G. death

1. Adenoviral vectors are better to use for killing cells (as in case of cancer gene therapy) than to cure a disease

2. Dose escalation studies should be better controlled

3. Completely gutless vectors should be used

Death of Jesse Gelsinger in a gene therapy trial at the University of Pennsylvania in the year 1999

Raper SE, Chirmule N, Lee FS, Wivel NA, Bagg A, Gao GP, Wilson JM, Batshaw ML: **Fatal systemic inflammatory response syndrome in a ornithine transcarbamylase deficient patient following adenoviral gene transfer.**
Mol Genet Metab 2003, 80: 148-158.

HUMAN GENE THERAPY 13:1 (January 1, 2002)
Mary Ann Liebert, Inc.

Editor's Note

Adenoviral Vector Safety and Toxicity

The Report discusses the background events that led to its writing, initiated by the death of Jesse Gelsinger in September, 1999.

Gene therapy of SCID patients in Europe
by retroviral gene transfer

A retroviral vector was used to 'infect' bone-marrow stem cells taken from each patient before being injected back into their bloodstream, where it was hoped they would multiply into normal immune cells.

The successful treatment of the first patients was greeted with excitement when it was first reported in 2000 and 2002

Cavazzana-Calvo, M. *et al*. *Science* 288, 669–672 (2000)
Aiuti, A. *et al*. *Science* 296, 2410–2413 (2002).

Results of X-SCID gene therapy

Alain Fischer at Necker Hospital, Paris

3,5 years after stem cell gene therapy, these X-SCID children (14 out of 15)

- are able to live normal lives at home instead of inside a sterile "bubble";
- have normal numbers of T cells of both the CD4 and CD8 subsets;

- have responded to several childhood immunizations, including diphtheria, tetanus and polio by producing both T cells and antibodies specific for these agents.

- Antibody production is sufficiently good that they have no need for periodic infusions of immunoglobulin (IG).

The failure:

Two of the ten children treated in France developed leukaemia-like conditions

The optimism generated by what was considered to be the first true success of gene therapy turned into disappointment at the news of the two leukaemia cases.

Genetic analysis of the malignant cells showed that in both cases the retroviral vector had inserted into, and activated, an oncogene called *LMO2* that is associated with childhood leukaemia.

The activated oncogene was not the only cause of the malignancy, but was most likely the event that triggered

Hacein-Bey-Abina, S. *et al*. *Science* **302**, 415–419 (2003).

Hacein-Bey-Abina S et al., **A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency.** *N Engl J Med 2003,* ***348:255-256.***

Gene therapy put on hold as third child develops cancer

Nature **433**, 561 (10 February 2005)

The first trial to be stopped was halted in October 2002, and other trials were halted three months later, after two children in the trials developed cancer. But authorities allowed them to resume during the past year because the treatment had cured many children who lack reliable alternative treatments

But on 24 January 2005, the French medical regulatory authority AFSSPS announced that a child who was treated by Fischer in April 2002 now has cancer.

Commentary

Arthritis gene therapy's first death

Christopher H Evans[1], Steven C Ghivizzani[2] and Paul D Robbins[3]

In July 2007, a recombinant AAV named tgAAC94, expressing a fusion protein consisting of the extracellular domain of human tumor necrosis factor receptor type II and the Fc domain of IgG1 (TNFR:Fc) was injected to a 36 year old woman suffering from rheumatodi arthritis locally into symptomatic joints with the expectation that the protein will be produced intraarticularly and will confer a local therapeutic effect.

However, she died 22 days after receiving a second dose. The study was placed on clinical hold while the circumstances surrounding this tragedy were investigated.

Early in December 2007, the Food and Drug Administration removed the clinical hold, allowing the study to resume with minor changes to the protocol.

http://arthritis-research.com/content/pdf/ar2411.pdf

Positive developments

http://www.cancertherapychina.com/index.php?option=com_content&view=article&id=84&Itemid=23

Gendicine™

Recombinant Human Adenovirus expressing p53

It is injected into the patients suffering from head and neck squamous cell carcinoma (HNSCC).

Professor Zhang Shanwen of the Beijing Cancer Hospital completed clinical trials II and the drug license was issued on October 16th, 2003 by the State Food and Drug Administration of China,

This is the world's first anticancer gene therapy drug.

Gendicine: the first commercial gene therapy product.

Wilson JM ***Hum Gene Ther 2005, 16:1014-1015.***

GENE THERAPY FOR PARKINSON'S DISEASE

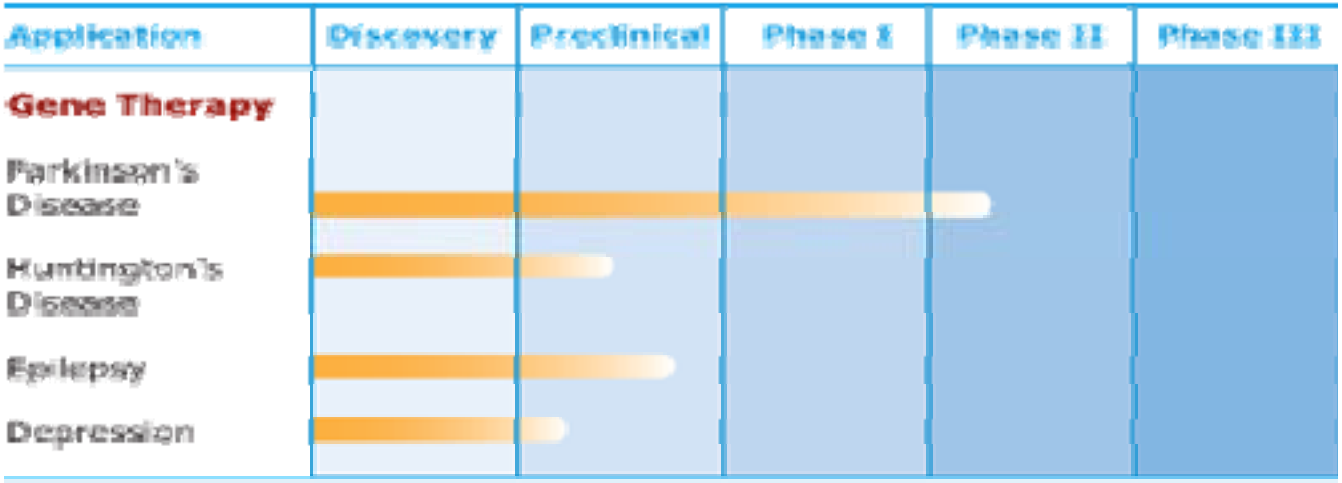
In Parkinson's disease, patients lose dopamine-producing brain cells, resulting in substantial reductions in the activity and amount of GABA (gamma-aminobutyric acid), the major inhibitory neurotransmitter in the brain.

This contributes to an abnormal increase in activity of the subthalamic nucleus (STN) of the brain, a key regulatory center for movement, and causes a dysfunction in brain circuitry responsible for coordinating movement.

GENE THERAPY FOR PARKINSON'S DISEASE

Neurologix, Inc.

Condition	Intervention	Phase
Parkinson's Disease	Gene therapy involving Bilateral surgical infusion of AAV-GAD into the subthalamic nucleus	Phase II



Neurologix Announces Successful Phase 2 Trial of Gene Therapy for Parkinson's Disease

FORT LEE, N.J., June 22, 2010 /PRNewswire via COMTEX/ -- Neurologix, Inc. (OTC Bulletin Board: NRGX), today announced positive results in a Phase 2 trial of its investigational gene therapy for advanced Parkinson's disease (PD), NLX-P101.

Study participants who received NLX-P101 experienced statistically significant and clinically meaningful improvements in off-medication motor scores compared to control subjects who received sham surgery.

In the trial, this benefit was seen at one month and continued virtually unchanged throughout the six month blinded study period. The results also demonstrated a positive safety profile for NLX-P101, with no serious adverse events related to the gene therapy or surgical procedure reported.

Patients enrolled in the trial had moderate to advanced PD and were not adequately responsive to current therapies.

Vol 467|16 September 2010 nature

NEWS & VIEWS

GENE THERAPY

Targeting β-thalassaemia

Derek A. Persons

nature Vol 467|16 September 2010|doi:10.1038/nature09328

LETTERS

Transfusion independence and *HMGA2* activation after gene therapy of human β-thalassaemia

Cavazzana-Calvo M et al., NATURE 467, 16, September 2010

In 2007, Cavazzana-Calvo and colleagues treated an 18-year-old male patient who had HbE/β-thalassaemia — a form of the disorder in which haemoglobin production is severely compromised. They treated the patient's hematopeitic stem cells (HSCs) with an HIV-derived lentiviral vector containing a functional β-globin gene. Thirty three months after lentiviral β-globin gene transfer, this patient with severe b^E/b^0-thalassaemia dependent on monthly transfusions since early childhood has become transfusion independent for 21 months after gene therapy. Blood haemoglobin was maintained between 9 and 10 g dl^{-1}, of which one-third contained vector-encoded β-globin.

HUMAN GENE THERAPY 21:915–927 (August 2010)

DOI: 10.1089/hum.2010.041

Review

Ocular Gene Therapy: An Evaluation of Recombinant Adeno-Associated Virus-Mediated Gene Therapy Interventions for the Treatment of Ocular Disease

Kamolika Roy, Linda Stein, and Shalesh Kaushal

rAAV-Mediated Gene Therapy for Retinal Degenerative Disorders

CONCLUSIONS

It would be unrealistic not to expect gene therapies to produce side effects.

Gene therapy continues to require informed use in controlled clinical studies with a clear consideration of the risks and potential benefits.

Current vector systems may need to be modified, and additional efforts are required to better understand the biology of the diseases that are candidates for therapeutic genetic intervention.

Together this information will enable risk classifications for specific vectors and transgenes, as well as assessment of the risk factors that are unique to each clinical trial.

With this approach, the therapeutic potential of somatic gene transfer may be realized through the application of appropriate prevention strategies.

Gene therapy resources

The **National Gene Vector Laboratory (NGVL)** is a US National Institutes of Health initiative charged with providing clinical grade vectors for gene therapy trials.

http://www.ngvl.org/

There are three production sites:
Baylor College of Medicine supplies adenoviral vectors,
The City of Hope Medical Center is the site for production of plasmid vectors and Indiana University generates retroviral and lentiviral vectors.
Two additional sites, the University of Florida and Southern Research Institute, supply toxicology studies in support of gene therapy clinical trials.

The **National Gene Vector Biorepository (NGVB)** is located at the Department of Medical and Molecular Genetics at the Indiana University School of Medicine

www.ngvbcc.org

Gene Therapy (2005) **12,** S28–S35.
Retroviral vector production in the National Gene Vector Laboratory at Indiana University
K Cornetta, L Matheson and C Ballas

http://www.nature.com/gt/index.html

Review articles appearing in gene therapy

http://www.nature.com/gt/progress_and_prospects.html

Essential topics explored in depth
in
Gene Therapy Special Issues

http://www.nature.com/gt/special_issues.html

http://www.liebertonline.com/toc/hum

http://www.nature.com/cgt/index.html

GENE THERAPY: Twenty-First Century Medicine

Inder M. Verma and Matthew D. Weitzman

Annu. Rev. Biochem. 2005. 74:711–38

Review articles

Progress and prospects: human artificial chromosomes
S Macnab and A Whitehouse *Gene Therapy* 16, 1180-1188 (October 2009)

Progress and prospects: Zinc-finger nucleases as gene therapy agents
Zinc-finger nucleases as gene therapy agents
D Carroll *Gene Therapy* 15, 1463-1468 (November 2008)

Progress and Prospects: targeted gene alteration (TGA)Targeted gene alteration
H Parekh-Olmedo and E B Kmiec *Gene Therapy* 14, 1675-1680 (December 2007)

Progress and Prospects: Gene Therapy Clinical Trials (Part 2)
U Griesenbach *Gene Ther*apy 14: 1555-1563 (November 2007)

Progress and Prospects: Gene Therapy Clinical Trials (Part 1)
Eric Alton *Gene Ther*apy 14: 1439-1447 (October 2007)

Progress and prospects: gene transfer into embryonic stem cells
F Yates and G Q Daley *Gene Therapy* 13: 1431-1439 (October 2006)

Review articles

Anderson WF. Gene therapy. *Scientific American. Sept 1995;124-8.*

Mulligan RC. The basic science of gene therapy. *Science 1993;260:926-30.*

Smith AE. Viral vectors in gene therapy. *Ann Rev. Microbiol. 1995;49:807-38.*

Harris JD, Lemoine NR. Strategies for directed gene therapy. *TIG. 1996;12:*

Miller N, Vile R. Targeted vectors for gene therapy. *FASEB J. 1995;9:190-9.*

Felgner PL. Nonviral strategies for gene therapy. *Scientific American. Jun 1997;102-6.*

S Li and L Huang (2000) Nonviral gene therapy: promises and challenges Gene Therapy 2000, 7: 31-34

Gene therapy using non viral vectors

First report of direct gene transfer into skeletel muscle using naked plasmid DNA.

Wolff JA *et al*.
Direct gene transfer into mouse muscle *in vivo*.
Science 1990; **247:** 1465-1468,

Plasmid DNA transfection using Gene gun and elecroporation

Liu F, Song Y, Liu D. Hydrodynamics-based transfection in animals by systemic administration of plasmid DNA. *Gene Therapy* 1999; **6:** 1258-1266

Yang NS *et al*. In vivo and *in vitro* gene transfer to mammalian somatic cells by particle bombardment. *Proc Natl Acad Sci USA* 1990; **87:** 9568-9572,

Rols MP *et al*. In vivo electrically mediated protein and gene transfer in murine melanoma. *Nat Biotechnol* 1998; **16:** 168-171

Rizzuto G *et al*. Efficient and regulated erythropoietin production by naked DNA injection and muscle electroporation. *Proc Natl Acad Sci USA* 1999; **96:** 6417-6422

Plasmid DNA transfection using cationic lipids

Felgner PL *et al*. Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure. *Proc Natl Acad Sci USA* 1987; **84:** 7413-7417

Wheeler CJ *et al*. A novel cationic lipid greatly enhances plasmid DNA delivery and expression in mouse lung. *Proc Natl Acad Sci USA* 1996; **93:** 11454-11459

Lee ER *et al*. Detailed analysis of structures and formulations of cationic lipids for efficient gene transfer to the lung. *Hum Gene Ther* 1996; **7:** 1701-1717

Cationic lipid-mediated gene transfer for the treatment of cancer and cystic fibrosis

Nabel GJ *et al*. Direct gene transfer with DNA-liposome complexes in melanoma: expression, biologic activity, and lack of toxicity in humans.
Proc Natl Acad Sci USA 1993; **90:** 11307-11311

Caplen NJ *et al*. Liposome-mediated CFTR gene transfer to the nasal epithelium of patients with cystic fibrosis. *Nat Med* 1995; **1:** 39-46