HC70A & SAS70A Winter 2014
Genetic Engineering in Medicine, Agriculture, and Law

Professors Bob Goldberg, Channapatna Prakash & John Harada

Lecture 8
Human Genetic Engineering and Gene Therapy

THEMES
Human Genetic Engineering and Gene Therapy
1. What is Gene Therapy?
   a. Germ Line
   b. Somatic Cell
2. Two Types of Somatic Cell Gene Therapy
   a. Ex Vivo Gene Therapy
   b. In Vivo Gene Therapy
3. Case Study: Ex Vivo Gene Therapy for Severe Combined Immunodeficiency (SCID)
4. Some Problems and Improvements with Gene Therapy
5. Other Examples of Ex Vivo Gene Therapy
6. In Vivo Gene Therapy
7. Targeted Killing of Specific Cell Types
8. Regulations and Issues Concerning Gene Therapy
Genetically Engineered Organisms & Their Uses

1. Bacteria
   a. Drugs

2. Fungi
   a. Drugs
   b. Fermentation

3. Animals
   a. Mouse Model-Knock-Outs-Human Gene Functions
   b. Farm Animals-Drugs

4. Plants
   a. Genetically Engineered Crops
   b. Feedstock for Biofuels
What is Gene Therapy?

- The insertion of usually genetically altered genes into cells especially to replace defective genes in the treatment of genetic disorders or to provide a specialized disease-fighting function - Merriam-Webster Dictionary
- Experimental treatment of a genetic disorder by replacing, supplementing, or manipulating the expression of abnormal genes with normally functioning genes - National Center for Biotechnology
- It is an approach to treating disease by either modifying the expressions of an individual’s genes or correction of abnormal genes - American Society of Gene and Cell Therapy
- Gene therapy is the use of DNA as a pharmaceutical agent to treat disease - Wikipedia

Types of Gene Therapy

- Germline gene therapy

- Somatic gene therapy
  - Gene supplementation
  - Gene replacement
  - Gene alteration
  - Targeted killing of specific cell-types
  - Targeted inhibition of gene expression

- Issues
  - Regulation
  - NIH Guidelines
  - Human Experimentation
  - Ethics
  - Eugenics
21.4 Principles of gene therapy

Gene therapy involves the direct genetic modification of cells of the patient in order to achieve a therapeutic goal. There are basic distinctions in the types of cells modified, and the type of modification effected.

1. Germ-line gene therapy produces a permanent transmissible modification. This might be achieved by modification of a gamete, a zygote or an early embryo. Germ-line therapy is banned in many countries for ethical reasons (see Ethic Box 2).

2. Somatic cell gene therapy aims to modify specific cells or tissues of the patient in a way that is confined to that patient. All current gene therapy trials and protocols are for somatic cell therapy.

   Somatic cells might be modified in a number of different ways (Figure 21.4).

   a. Gene supplementation (also called gene augmentation) aims to supply a functioning copy of a defective gene. This would be used to treat loss-of-function conditions (Section 16.6) where the disease process is the result of a gene not functioning here and now. Cystic fibrosis would be a typical candidate. It would not be suitable for loss-of-function conditions where irreversible damage has already been done, for example through some failures in embryonic development. Cancer therapy could involve gene supplementation to increase the immune response against a tumor or to replace a defective tumor suppressor gene.

   b. Gene replacement is more ambitious: the aim is to replace a mutant gene by a correctly functioning copy, or to correct a mutation in it. Gene replacement would be required for gain-of-function diseases where the resident mutant gene is doing something positively bad.

   c. Targeted inhibition of gene expression is especially relevant in infectious disease, where essential functions of the pathogen are targeted. It could also be used to silence acquired oncogenes in cancer, to damp down unavailing response to autoimmune disease and maybe to silence a gain-of-function mutant allele in inherited disease.

   d. Targeted killing of specific cells is particularly applicable to cancer treatment.

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Which type(s) of gene therapy should be allowed?

a. Germline cell gene therapy
b. Somatic cell gene therapy
c. Both
d. Neither
The following type(s) of gene therapy can be conducted legally in the United States.

a. Germline cell gene therapy  
b. Somatic cell gene therapy  
c. Both  
d. Neither

Questions to Consider Before Initiating Gene Therapy

1. Does the condition result from a mutation of one or more genes?  
2. What is known about the biology of the disorder?  
3. Has the gene been cloned?  
4. Will adding a normal copy of the gene fix the problem in the affected tissue?  
5. Can you deliver the gene to cells of the affected tissue?
**In Vivo and Ex Vivo Gene Therapy**

**Ex Vivo Gene Therapy**

Ex vivo gene therapy is performed with the genetic alterations of patient's target cells happening outside of the body in a culture. Target cells from the patient are infected with a recombinant virus containing the desired therapeutic gene. These modified cells are then reintroduced into the patient's body, where they produce the needed proteins that correspond to the inserted gene.

1. copies of therapeutic gene
2. gene inserted into viral DNA
3. cultured cells infected with genetically-altered virus
4. patient's sample target cells are now genetically altered with therapeutic gene
5. cells are reintroduced into body

Inside the body, the genetically altered cells produce the desired proteins encoded by the therapeutic DNA.
**In Vivo Gene Therapy**

In vivo gene therapy involves introduction of therapeutic DNA directly into the patient's body. The DNA is introduced by cell-specific direct injection into tissue in need. DNA in the form of a plasmid vector is introduced by a dermal vaccination. Modified liposomes are not currently used for gene therapy, but they will likely be the next advancement in therapeutic gene delivery as cell-specific receptor-mediated DNA carriers. Once inside the body and in contact with the specifically targeted cells, the inserted DNA is incorporated into the tissue's cells where it encodes the production of the needed protein.

**Case Study of Ex Vivo Gene Therapy for Severe Combined Immunodeficiency (SCID)**
Severe Combined Immunodeficiency (SCID) Disease

Adenosine Deaminase Gene (ADA) Deficiency

- 32,213 kb Gene
- Chromosome 20
- 12 Exons
- 1,092 kb mRNA
- 323 aa protein

Degradation of Purine

- ADA is an enzyme that metabolizes adenosine and deoxyadenosine
- ADA deficiency results in elevated adenosine and deoxyadenosine levels
- Abnormal levels impair lymphocyte development and function
- The immune system is severely compromised or completely defective
- ADA deficiency accounts for ~15% of all SCID cases
- SCID-ADA patients can be treated with PEG-ADA, a stabilized form of the enzyme

Humans Have Been Genetically Engineered To Cure a Lethal Genetic Disease (SCID)

Gene therapy cures 'bubble boy disease'

31 Jan 2009, 1128 hrs IST, AP

The Age of Human Genetic Engineering Began More than Twenty Years Ago - SCID Treated with a Normal ADA Gene!!!

Several People are Alive Because They Have Been Engineered With an ADA Gene

Gene Therapy for Immunodeficiency Due to Adenosine Deaminase Deficiency

Gene Therapy with the Adenosine Deaminase (ADA) Gene
Ex Vivo Gene Therapy for Severe Combined Immunodeficiency (SCID)

1. Bacterium carrying plasmid with cloned normal human ADA gene
2. Genetically disabled retrovirus
3. T cells with disabled ADA gene isolated from SCID patient
4. Cloned ADA gene is incorporated into virus
5. Retrovirus infects T cells, transfers ADA gene to cells
6. Cells are grown in culture to ensure ADA gene is active

Vectors Used to Deliver Genes to Cells in Gene Therapy

<table>
<thead>
<tr>
<th>Vector</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retrovirus</td>
<td>Efficient transfer</td>
<td>Transfers DNA only to dividing cells, inserts randomly; risk of producing wild-type viruses</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>Transfers to nondividing cells</td>
<td>Causes immune reaction</td>
</tr>
<tr>
<td>Adeno-associated virus</td>
<td>Does not cause immune reaction</td>
<td>Holds small amount of DNA; hard to produce</td>
</tr>
<tr>
<td>Herpes virus</td>
<td>Can insert into cells of nervous system; does not cause immune reaction</td>
<td>Hard to produce in large quantities</td>
</tr>
<tr>
<td>Lentivirus</td>
<td>Can accommodate large genes</td>
<td>Safety concerns</td>
</tr>
<tr>
<td>Liposomes and other</td>
<td>No replication; does not stimulate immune reaction</td>
<td>Low efficiency</td>
</tr>
<tr>
<td>Direct injection</td>
<td>No replication; directed toward specific tissues</td>
<td>Low efficiency; does not work well within some tissues</td>
</tr>
<tr>
<td>Pressure treatment</td>
<td>Safe, because tissues are treated outside the body and then transplanted into the patient</td>
<td>Most efficient with small DNA molecules</td>
</tr>
<tr>
<td>Gene gun (DNA coated on small gold particles and shot into tissue)</td>
<td>No vector required</td>
<td>Low efficiency</td>
</tr>
</tbody>
</table>

Comparison of Virus and Cell Sizes

Note: $1 \text{ nm} = 10^{-9} \text{ m}$
Human Retroviruses Are Used As Gene Therapy Vectors

<table>
<thead>
<tr>
<th>Disease</th>
<th>Pathogen</th>
<th>Genome</th>
<th>Vector/Epidemiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis B (viral)</td>
<td>Hepadnavirus</td>
<td>Double-stranded DNA</td>
<td>Highly infectious through contact with infected body fluids. Approximately 1% of U.S. population infected. Vaccine available. No cure. Can be fatal.</td>
</tr>
<tr>
<td>Herpes</td>
<td>Herpes simplex virus</td>
<td>Double-stranded DNA</td>
<td>Blisters spread primarily through skin-to-skin contact with cold sores/listers. Very prevalent worldwide. No cure. Exhibits latency—the disease can be dormant for several years.</td>
</tr>
<tr>
<td>Mononucleosis</td>
<td>Epstein-Barr virus</td>
<td>Double-stranded DNA</td>
<td>Spreads through contact with infected saliva. May last several weeks common in young adults. No cure. Rarely fatal.</td>
</tr>
<tr>
<td>Smallpox</td>
<td>Variola virus</td>
<td>Double-stranded DNA</td>
<td>Historically a major killer; the last recorded case of smallpox was in 1977. A worldwide vaccination campaign wiped out the disease completely.</td>
</tr>
<tr>
<td>AIDS</td>
<td>HIV</td>
<td>(+) Single-stranded RNA (two copies)</td>
<td>Destroys immune defense, resulting in death by infection or cancer. As of 2020, WHO estimated that 40 million people are living with AIDS; 4.1 million new HIV infections were predicted and 2.8 million deaths were expected. More than 25 million have died from AIDS since 1981.</td>
</tr>
<tr>
<td>Polio</td>
<td>Enterovirus</td>
<td>(+) Single-stranded RNA</td>
<td>Acute viral infection of the CNS that can lead to paralysis and is often fatal. Prior to the development of Salk’s vaccine in 1954, 60,000 people a year contracted the disease in the U.S. alone.</td>
</tr>
</tbody>
</table>

HIV is a Retrovirus

T-Cell
Discovery of Retroviruses

Rous Sarcoma Virus is a Retrovirus That Causes Cancer and Contains Oncogenes in its Genome
Francis Peyton Rous Nobel Prize, 1966

Reverse Transcriptase is Encoded by a Retrovirus Genome and Converts the RNA Genome into a Double-Stranded DNA that is Integrated Into the Host Cell Genome

Figure 5-72 Molecular Biology of the Cell (© Garland Science 2008)
Retrovirus Life Cycle

Retroviruses Replicate Using Reverse Transcriptase
David Baltimore & Howard Temin—Nobel Prize 1975
Modified the Central Dogma of Molecular Biology
Use For Genetic Engineering?

Retrovirus Genome

5'LTR PBS accessory genes PPT 3'LTR
U3 R U5 gag pro pol env U3 R U5

env Surface Glycoprotein SU gp 120
denv Transmembrane Glycoprotein TM gp41
gag Membrane Associated (Matrix) Protein MA p17
gag Capsid CA (Core Shell) p24
capsid Reverse Transcriptase, RNA
polymerase PR p6
Integrase IN p32
**Retroviral Vector**

- **PSI** = Packaging Sequence
- **GAG** = Capsid Protein
- **POL** = Reverse Transcriptase
- **ENV** = Envelope Protein

**Retroviral Gene Therapy Vector**

**Using a Retrovirus as a Vector For Human Ex Vivo Gene Therapy**

- **Gag** = Capsid Protein
- **Pol** = Reverse Transcriptase
- **Env** = Envelope Protein
- **Ψ (Psi)** = Packaging Sequence
Using Retroviruses for Ex Vivo Gene Therapy

1. Cloning in Bacteria
2. DNA Transformation into Packaging Cell

Packaging Cell Line (Made Previously)

A. 1. Packaging Cells Makes Viral Proteins
   2. Cannot Package (Ψ-Minus)
   3. Packages Therapeutic Transcript (Ψ-Plus)

1. Infect Target Cells
2. Check For Presence of Gene
3. Transfer To Patient

RETROVIRAL VECTORS are assembled, or packaged, in cells designed to release only safe vectors. Investigators substitute a therapeutic gene for viral genes in a provirus (a) and insert that provirus into a packaging cell (b). The viral DNA directs the synthesis of viral RNA but, lacking viral genes, cannot give rise to the proteins needed to package the RNA into particles for delivery to other cells. The missing proteins are supplied by a “helper” provirus from which the psi region has been deleted. Psi is crucial to the inclusion of RNA in viral particles; without it, no virus carrying helper RNA can form. The particles that escape the cell, then, carry therapeutic RNA and no viral genes. They can enter other cells (c) and splice the therapeutic gene into cellular DNA, but they cannot reproduce.

Did the Gene Therapy Strategy Work?

T Lymphocyte-Directed Gene Therapy for ADA\textsuperscript{−} SCID: Initial Trial Results After 4 Years

R. Michael Blaese, Kenneth W. Culver, Dusty Miller, Charles S. Carter, Thomas Fleisher, Mario Ciceri, Gene Shearer, Lauren Chang, Yawen Chiang, Paul Tolstoshev, Jay J. Greenblatt, Steven A. Rosenberg, Harvey Klein, Melvin Berger, Craig A. Mullen, W. Jay Ramsey, Linda Muul, Richard A. Morgan, W. French Anderson

In 1990, a clinical trial was started using retroviral-mediated transfer of the adenosine deaminase (ADA) gene into the T cells of two children with severe combined immunodeficiency (ADA\textsuperscript{−} SCID). The number of blood T cells normalized as did many cellular and humoral immune responses. Gene treatment ended after 2 years, but integrated vector and ADA gene expression in T cells persisted. Although many components remain to be perfected, it is concluded here that gene therapy can be a safe and effective addition to treatment for some patients with this severe immunodeficiency disease.

- ADA gene expression in T cells persisted after four years
- Patients remained on ADA enzyme replacement therapy throughout the gene therapy treatment

Ashanthi DeSilva
Gelsinger had a mild form of ornithine transcarbamylase deficiency – results in an inability to metabolize ammonia.

He volunteered for clinical trial of gene supplementation therapy and was injected with adenovirus vector containing OTC gene.

He died of systemic inflammatory response syndrome - immune reaction to adenovirus vector.

3 of 17 patients in clinical trial for SCID gene therapy developed clonal lymphoproliferative disorder – a leukemia.

The leukemia was caused by insertion of retrovirus near proto-oncogenes and activation of these proto-oncogenes by retroviral switches.

Some Problems with Human Gene Therapy

- Delivery systems to target cells
- Expression levels of therapeutic gene
- Adverse immune reactions to vector
- Insertional mutagenesis causing other diseases (e.g., leukemia)
- Human error - failure to adhere to strict NIH and IRB procedures (experimental therapies)
A Comeback for Gene Therapy

Gene Therapy for Immunodeficiency Due to Adenosine Deaminase Deficiency

The New York Times

Giving Sight by Therapy With Genes

ScienceDaily

Gene therapy for red-green colour blindness in adult primates

LETTERS

Improvements in Gene Therapy

- Increases in efficiency of viral transduction
- Higher levels of therapeutic gene expression
- Development of self-inactivating vectors
- Coupling of gene therapy and stem cell technologies
Dr. Pei-Yun Lee

General Strategy for Use of Hematopoietic Stem Cells in Gene Therapy

1. Patient with MLD or WAS
2. Highly purified, high-titer LV
3. Cell release without cryopreservation
4. Myelosuppression (± immunosuppression adapted to specific disease)

Gene therapy with hematopoietic stem cells targets specific diseases through the introduction of corrected genes and the use of cytokines to stimulate cell growth.
Updated Ex-Vivo Gene Therapy for ADA-SCID & SCID-X1

- **SCID-X1**
  - Most common form of SCID
  - Results from mutations in the common gamma chain gene required for interleukin receptors
  - Patients are immune deficient

- **Gene Therapy Improvements**
  - Used hematopoietic stem cells
  - Improved retroviral vectors with higher titers

*How It Works* | The procedure the SCID-X1 trial will use
---|---
Bone marrow cell | Normal gene

Results after 10 years
- ADA-SCID - 4 of 6 children experienced immune reconstitution
- SCID-X1 - 9 of 10 children experienced normal T-cell number
- In another study, 5 of 20 SCID-X1 subjects experienced leukemia-like T lymphoproliferation
Development of Self-Inactivating (SIN) Vectors

1. First generation vectors often caused leukemia because they inserted viral DNA next to proto oncogenes.

2. The 5' LTR of the viral vector is a powerful switch that can activate proto oncogenes and cause cancers to form.

3. SIN vectors have transcriptionally disabled LTRs. They do not activate adjacent genes.

“Eight of the nine boys registered to date in the new trial are alive and well, with functioning immune systems and free of infections associated with SCID-X1, between nine and 36 months following treatment”.

**X-linked severe combined immunodeficiency syndrome: Gene therapy trial shows promising early results**

**Date:** December 9, 2013

**Source:** Dana-Farber/Boston Children's Cancer and Blood Disorders Center

**Summary:** Researchers reported promising outcomes data for the first group of boys with X-linked severe combined immunodeficiency syndrome, a fatal genetic immunodeficiency also known as "bubble boy" disease, who were treated as part of an international clinical study of a new form of gene therapy. Its delivery mechanism was designed to prevent the leukemia that arose a decade ago in a similar trial in Europe.
Other Examples of Ex Vivo Gene Therapy Using Hemotopoietic Stem Cells

Hemoglobin & Blood Oxygen Transport

Circulatory System

Red Blood Cells and Oxygen Transport

Hemoglobin Binds Oxygen
Ex-vivo Gene Therapy for β-Thalassemia

- β-Thalassemia results from a recessive mutation in the β-globin gene that causes reduced rates of synthesis and causes formation of abnormal hemoglobin and anemia.
- Disease is treated with regular blood transfusions.

Gene therapy - transduced hematopoietic stem cells (HSC) with lentivirus (HIV) engineered with β-globin gene.
- Transplanted therapeutic HSCs into patient following chemotherapy to destroy diseased HSCs.
- Patient has not needed transfusions for two years.

Gene Therapy
- Used hematopoietic stem cells (HSC) from patient.
- Used lentivirus vector with a β-globin gene engineered to impede sickle hemoglobin polymerization.
- Showed a lower percentage of the transduced red blood cells exhibited sickling compared with control cells from sickle donors.
In Vivo Gene Therapy

Blindness – Choroideremia (CHM)

1. CHM is a rare inherited cause of blindness that affects around 1 in 50,000 people. Night blindness is an early symptom.
2. CHM is caused by mutation in the X-linked REP1 gene.
3. Without the REP1 protein, pigment cells in the retina die prematurely.
Gene therapy 'could be used to treat blindness'

Adeno-associated viruses (AAV)
- Does not generally provoke antibody formation
- Infests nondividing cells of many different tissues
- Little or no integration of viral DNA into the host genome

Jonathan Wyatt, one of six patients whose vision improved as a result of REP1 gene therapy

LCA Gene Therapy Using RPE65 & AAV2

Leber Congenital Amaurosis
- Degenerative diseases of the retina
- The most common cause of congenital blindness in children

Type 2 LCA is caused by recessive mutations in the RPE65 isomerase gene that recycles photoreceptors

SUCCESS!

ALESSANDRO CANNATA

Cideciyan et al. PNAS 2008;105:15112
## Target for in Vivo Gene Therapy: Hemophilia B that is Caused by Mutations in Factor IX Gene

### Table 13.2: Some Important Genetic Disorders

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Symptom</th>
<th>Defect</th>
<th>Dominant/Recessive</th>
<th>Frequency Among Human Births</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystic fibrosis</td>
<td>Airways clog lungs, liver, and pancreas</td>
<td>Failure of chloride ion transport mechanism</td>
<td>recessive</td>
<td>1/2,500 (Caucasians)</td>
</tr>
<tr>
<td>Sickle cell anemia</td>
<td>Blood circulation is poor</td>
<td>Abnormal hemoglobin molecules</td>
<td>recessive</td>
<td>1/1000 (African Americans)</td>
</tr>
<tr>
<td>Early-onset disease</td>
<td>Central nervous system deteriorates in infancy</td>
<td>Defective enzyme (transaminase A)</td>
<td>recessive</td>
<td>1/1500 (Ashkenazi Jews)</td>
</tr>
<tr>
<td>Phenylketonuria</td>
<td>Brain fails to develop in infancy</td>
<td>Defective enzyme (phenylalanine hydroxylase)</td>
<td>recessive</td>
<td>1/12,000</td>
</tr>
<tr>
<td>Hemophilia</td>
<td>Blood fails to clot</td>
<td>Defective blood-clotting factor VIII</td>
<td>X-linked recessive</td>
<td>1/10,000 (Caucasian males)</td>
</tr>
<tr>
<td>Huntington disease</td>
<td>Brain tissues gradually deteriorate in middle age</td>
<td>Production of an inhibitor of brain cell metabolism</td>
<td>dominant</td>
<td>1/2,500</td>
</tr>
<tr>
<td>Muscular dystrophy</td>
<td>Muscles waste energy</td>
<td>Degradation of myelin covering nerves stimulating muscles</td>
<td>X-linked recessive</td>
<td>1/3700 (males)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>Excessive cholesterol levels in blood lead to heart disease</td>
<td>Abnormal form of cholesterol cell surface receptor</td>
<td>dominant</td>
<td>1/500</td>
</tr>
</tbody>
</table>

18,000 People in US Have Hemophilia & 400 Babies/Year Are Born With Disorder Prior to 1960s – Average Life Span Was 11 Years

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Defective Gene</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemophilia A</td>
<td>Defective Factor VIII Gene</td>
<td>1/10,000 males</td>
<td>80%</td>
</tr>
<tr>
<td>Hemophilia B</td>
<td>Defective Factor IX Gene</td>
<td>1/30,000 males</td>
<td>20%</td>
</tr>
<tr>
<td>Hemophilia C</td>
<td>Defective Factor XI Gene</td>
<td>Autosomal</td>
<td>&lt;1%</td>
</tr>
</tbody>
</table>

Both Factor VIII & IX Genes on X-Chromosome (♂ ♀ s)

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### How Does Blood Clot After Wounding?

**Post-translational Modification**

**Clothting Cascade:** Begins when cell damage at a wound somehow activates the enzyme factor XII, which then converts factors XII into fibronectin by thrombosis. At each step an inactive protein is converted into a protease or protein-cleaving enzyme (factor), which activates the next protein. Some steps require cofactors such as factors VIII and V. The cascade includes positive and negative feedback loops (positive arrows). Thrombin activates factors VIII and V, it also activates them (by activating protein C) which helps to halt clotting. Some 85 percent of hemophiliacs lack factor VIII. The rest lack factor IX.

What Happens If Any Of These Proteins Or Genes Are Mutated? **↓ No Blood Clot!**
Protocol
- Transferred Human Factor IX gene into adenovirus-associated virus vector that targets liver cells
- Infused AAV8 vector into six participants with severe hemophilia B (FIX <1% of normal)
- Participants monitored for 6-16 months

Results
- AAV-mediated expression of FIX at 2 to 11% of normal levels
- Four of six discontinued FIX prophylaxis; in the other two, the interval between prophylactic injections was increased

Targeted Killing of Specific Cell Types
Leukemia is cancer of the blood, that results in an increase in immature white blood cells. Chronic lymphoid leukemia affects B cell lymphocytes.
Ex-vivo Gene Therapy for Lymphocytic Leukemia

**Protocol**
- Removed T cells from patients
- Created Chimeric Antigen Receptor (CAR) genes that recognize a protein on the surface of B cells
- Transferred CAR genes into T cells to allow them to target B cells
- Infused CAR T cells back into patients

**Results**
- CAR T cells expanded more than 1,000 fold and persisted more than six months
- Estimated that each CAR T cell killed more than 1,000 cancer cells
- In one trial, 19 of 22 children who had exhausted all drug treatment and bone-marrow transplant options for leukemia went into remission after receiving CART-19
- 45 of 75 leukemia patients saw complete regressions with CARs

Current Status of Gene Therapy
Clinical Trials

- Phase I: Safety
  - Usually includes healthy (paid) volunteers

- Phase II: Efficacy
  - Patients are involved
  - Usually where drug fails

- Phase III---Randomized controlled trial
  - Involves larger numbers of patients
  - Compares efficacy of drug against current “gold standard” treatment
  - Expensive
Approved Gene Therapy Trials

- Cytokine 26% (n = 227)
- Antigen 14% (n = 128)
- Tumor suppressor 12% (n = 113)
- Suicide 6% (n = 74)
- Deficiency 7.8% (n = 68)
- Drug resistance 6.1% (n = 56)
- Receptor 3.4% (n = 31)
- Replication inhibitor 2.9% (n = 27)
- Others 14% (n = 129)
- N/C 6% (n = 55)

Figure 5. Distribution of gene therapy clinical trials by gene. N/C = not communicated

Table 1. Selected gene therapy clinical trials.

<table>
<thead>
<tr>
<th>Therapy Method</th>
<th>Disease</th>
<th>Phase of Development</th>
</tr>
</thead>
<tbody>
<tr>
<td>vector-mediated</td>
<td></td>
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</tr>
<tr>
<td>AAV based</td>
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<tr>
<td>Table:</td>
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</table>

Nature Biotechnology, February 2011
<table>
<thead>
<tr>
<th>Gene Therapy for genetic disease</th>
<th>Gene Therapy for congenital disease</th>
<th>Gene Therapy for cancer</th>
<th>Gene Therapy for other</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>Adenosine deaminase deficiency</td>
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Gendicine is a genetically engineered, infectious active recombinant human p53 adenovirus particles (rAd-p53), the replication-defective adenovirus type 5 and human p53 tumor suppressor gene normally composed of two parts, a replication-defective adenovirus particles as a carrier of the p53 gene into tumor cells, p53 gene expression in tumor cells of p53 protein plays inhibit tumor cell growth and induced apoptosis of tumor cells, inhibiting the biological function of tumor angiogenesis and bystander effects.

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**Approved Gene Therapy Products**

**uniQure**

Glybera® (lipogense tiparvovet) overview

Glybera is a gene therapy that is designed to restore the LPL enzyme activity required to enable the processing, or clearance, of fat-containing chylomicron particles formed in the intestine after a fat-containing meal. The product consists of an engineered copy of the human LPL gene packaged with a tissue-specific promoter in a non-replicating AAV1 vector, which has a particular affinity for muscle cells. In order to improve activity, uniQure uses a naturally occurring variant of the LPL gene that has higher enzyme activity than the normal version of the gene that encodes the protein. The company produces Glybera using its insect cell-based manufacturing process. Physicians administer Glybera in a one-time series of up to 60 intramuscular injections in the legs. The patient is administered spinal anesthesia or deep sedation during the procedure. In addition, an immunosuppressive regimen is recommended from three days prior to and for 12 weeks following Glybera administration.

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**Regulations and Issues Concerning Gene Therapy**
US Regulatory Authority for Gene Therapy

- Department of Health and Human Services (DHHS) has been charged with oversight of clinical trials through Code of Federal Regulations
  - Office for Human Research Protections
    - All research involving human subjects undergo Institutional Review Board review
  - U.S. Food and Drug Administration
    - Center for Biologics Evaluation and Research regulates human gene therapies. Manufacturers of gene therapy products must test their products extensively and meet FDA requirements for safety, purity and potency before they can be sold in the United States
- National Institutes of Health (NIH), oversees the conduct of federally funded clinical trials
  - Recombinant DNA Advisory Committee review human gene transfer research on behalf of the NIH through the Office of Biotechnology Activities

http://www.genetherapynet.com/united-states-of-america.html

FDA meeting to discuss “oocyte modification” in assisted reproduction for the prevention of transmission of mitochondrial disease – February 25-26, 2014

Agenda
On February 25, 2014, from 8 a.m. to 5:30 p.m. and on February 26, 2014, from 8 a.m. to approximately 11:15 a.m., the committee will discuss oocyte modification in assisted reproduction for the prevention of transmission of mitochondrial disease or treatment of infertility. On February 26, 2014, from approximately 11:15 a.m. to 11:30 a.m., the committee will hear updates on guidance documents issued from the Office of Cellular, Tissue, and Gene Therapies, Center for Biologics Evaluation and Research (CBER), FDA. On February 26, 2014, from 1 p.m. to approximately 5 p.m., the committee will discuss considerations for the design of early-phase clinical trials of cellular and gene therapy products. CBER published guidance on this topic in July 2013.
Would you alter a somatic cell of your child for the trait(s) of “your choice” using somatic cell gene therapy if the procedure was 100% “safe?”

a. Yes
b. No

Would you alter the germ line of your child for the trait(s) of “your choice” using germ-line gene therapy if the procedure was 100% “safe?”

a. Yes
b. No
We propose the following ethico-scientific criteria which any prospective techniques for gene therapy in human patients should satisfy:

1. There should be adequate biochemical characterization of the prospective patient's genetic disorder.
2. There should be prior experience with untreated cases of what appears to be the same genetic defect.
3. There must be an adequate characterization of the quality of the exogenous DNA vector.
4. There should be extensive studies in experimental animals to evaluate the therapeutic benefits and adverse side effects of the prospective techniques.
5. Where possible, determine whether the prospective gene therapy technique can restore enzyme function in the cells of the prospective patient.

Some Issues With Human Gene Therapy

- Regulation
- Consent
- Risks
- Enhancement
- Eugenics (Germ Line)
- Availability To Everyone
The Frontiers of Human Gene Therapy: RNAi “Drugs”, Vaccines, & Genome Editing

Gene Therapy for Dominant Mutations: a “Molecular Drug” to Shut Off Genes - RNAi

Lou Gehrig’s Disease - Amyotrophic Lateral Sclerosis (ALS)
- One cause is a dominant mutation in the coding region of the superoxide dismutase (SOD1) gene (SOD is an anti-oxidant)
- Mutant SOD1 Protein is Toxic to Motor Neurons

Amyloidosis
- Diseases in which normally soluble proteins become insoluble and deposited outside of cells in various tissues
- An inherited amyloidosis, abnormal transthyretin protein aggregates into amyloid fibrils in the liver, eventually causing death

If the mutant gene is shut off with a “Molecular Drug,” disease might not develop.
Small RNAs Target Specific mRNAs For Degradation and/or Protein Synthesis Inhibition

RNAi is Considered to be the Genome’s “Immune System” Protecting Against RNA Viruses & Transposable Element Movement

Andrew Fire & Craig Mello
Nobel Prize-2006

RNA Interference (RNAi) Specifically Inhibits the Accumulation of Targeted Proteins

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Using RNAi To Inhibit Gene Activity

Protocol
- Create a small interfering RNA (siRNA) against transthyretin (TTR) mRNA with a modified phosphodiester RNA backbone
- Encapsulate siRNA in lipid nanocarriers
- Deliver the drug intravenously

Results
- Observed a 82 - 87% mean reduction in TTR levels
- Efficiency of TTR knockdown supports monthly or bimonthly dosing
- No adverse effects observed
Molecular Drug for Muscular Dystrophy

**Duchenne Muscular Dystrophy**
- Results in intellectual disability, muscle weakness, and difficulty with motor skills
- Caused by defective dystrophin gene, X-linked gene that is the largest in the human genome, encompassing 2.6 million base pairs of DNA and containing 79 exons
- Absence of dystrophin leads to muscle fiber damage and membrane leakage
- In one form, the defective dystrophin gene has a deletion of Exon 50

![Dystrophin links the cell membrane with actin filaments](image)

RNAi-based Exon Skipping Treatment for Duchenne Muscular Dystrophy

**Protocol**
- Design a siRNA against Exon 51 in mutated dystrophin gene
- Create the siRNA with a modified phoshodiester backbone
- Inject the drug into muscle

**Results**
- 4 patients with the highest dose could walk 69 meters further in six minutes than control group
- Muscle fibers that tested positive for dystrophin increased 47%
**Vaccine for the Hepatitis C Virus**

**Hepatitis C**
- Caused by the Hepatitis C virus (HCV), a single-stranded RNA virus
- HCV infects the liver. Chronic infection can lead to cirrhosis, liver failure, or liver cancer
- Replication of HCV requires microRNA 122 (similar to siRNA)

**A Hepatitis C Vaccine**
- Create locked nucleic acid that targets micro RNA 122
- Administer intravenously
- Resulted in marked suppression of virus levels in chronically HCV-infected chimpanzees

**HCV Life Cycle**

**Promising Future of Human Gene Therapy**

*IN brief*
First gene therapy approved

*Phase Ia Clinical Evaluation of the Plasmodium falciparum Blood-stage Antigen MSP1 in ChAd63 and MVA Vaccine Vectors*

*NATURE MEDICINE | LETTER*
Gene therapy rescues cilia defects and restores olfactory function in a mammalian ciliopathy model

*SERCA2a Gene Therapy for Heart Failure: Ready for Primetime?*
Muthu Perlasamy and Anuradha Kalyanasundaram

*PLOS BLOGS*
Restoration of Hearing in the VGLUT3 Knockout Mouse Using Virally Mediated Gene Therapy
Combining Gene Therapy With Stem Cells & Therapeutic Cloning in the Future

Genetic Engineer Cells Before Nuclear or Cell Transfer

Example Defective Insulin Gene in Pancreas

Approaches to Specifically Edit the Genome

- DNA editing nucleases introduce double-stranded breaks in DNA
- DNA sequences can be specifically altered during the repair of these breaks
- Genes can be specifically targeted to become inactivated, altered, or added to the genome.
Human Genome Editing Therapy

The Future is Now for Human Genome Editing Therapy

Genomic Editing of the HIV-1 Coreceptor CCR5 in Adult Hematopoietic Stem and Progenitor Cells Using Zinc Finger Nucleases

Reading Frame Correction by Targeted Genome Editing Restores Dystrophin Expression in Cells From Duchenne Muscular Dystrophy Patients

Editing of Targeted Genes Proved Possible in Monkeys
The End!!

HC70A/SAS70A Lectures on the History, Science, and Applications of Genomics & Genetic Engineering

EXPERIMENT

HYPOTHESIS: Biologically functional recombinant chromosomes can be made in the laboratory.

METHOD

E. coli plasmids carrying a gene for resistance to either the antibiotic tetracycline or kanamycin are not with a restriction enzyme.

RESULTS

Some E. coli resistant to both antibiotics.

No E. coli resistant to both antibiotics.

CONCLUSION: Two DNA fragments with different genes can be joined to create a recombinant DNA molecule, and the resulting DNA is functional.