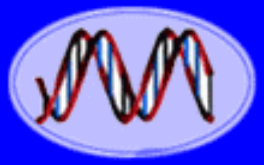
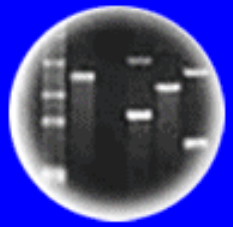


DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

HC70A & SAS70A Winter 2013
Genetic Engineering in Medicine, Agriculture, and Law

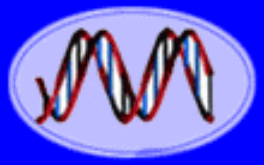
Professors Bob Goldberg, Channapatna Prakash & John Harada

Lecture 8
Human Genetic Engineering and Gene Therapy

UCLA

TUSKEGEE UNIVERSITY

UCDAVIS
UNIVERSITY OF CALIFORNIA



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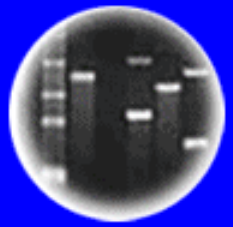


DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

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 with Human Gene Therapy
5. Another Example of Ex Vivo Gene Therapy
6. In Vivo Gene Therapy
7. Targeted Killing of Specific Cell Types
8. Issues With Human Gene Therapy
9. The Frontiers of Human Gene Therapy: RNAi "Drugs", Vaccines, Therapeutic Cloning + Gene Therapy

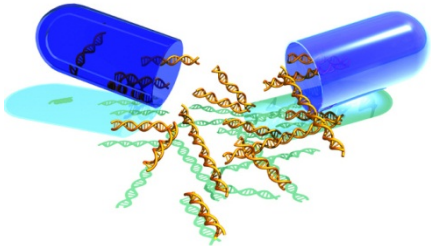
Genetically Engineered Organisms & Their Uses

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

Human Genetic Engineering and Gene Therapy

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*

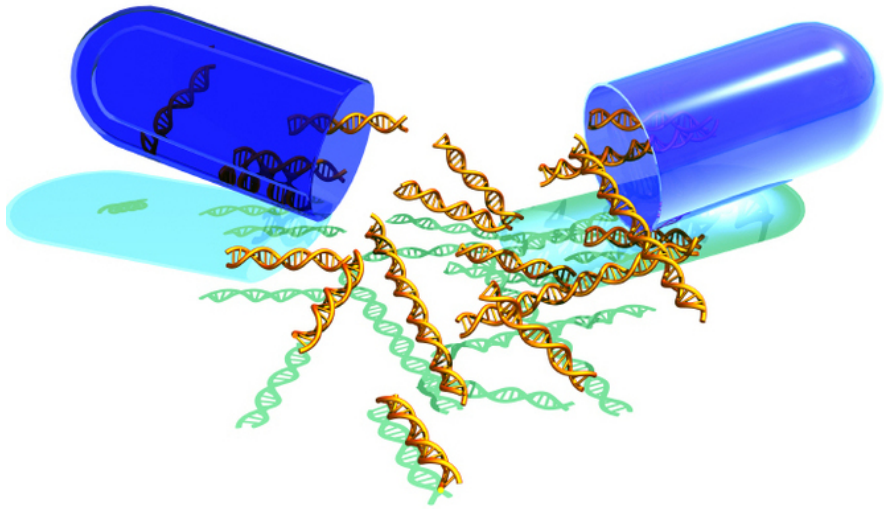


Types of Gene Therapy

- Germline gene therapy

- Somatic gene therapy
  - Gene supplementation
  - Gene replacement
  - 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 *Ethics 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*).

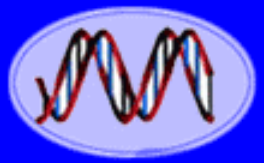
a. ▸ **Gene supplementation** (also called gene augmentation) aims to supply a functioning copy of a defective gene. This would be used to treat loss-of-function conditions (Section 16.4) where the disease process is the result of a gene not functioning here and now. Cystic fibrosis would be a typical candidate. It would not be suitable for loss-of-function conditions where irreversible damage has already been done, for example through some failure 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 situ*. 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 activated oncogenes in cancer, to damp down unwanted responses in 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.

Issues
Regulation?
NIH Guidelines?
Human Experimentation?
Ethics?
Eugenics?



DNA
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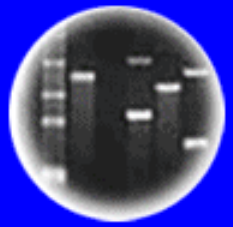


DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

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

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?

Ex Vivo vs In Vivo Somatic Cell Gene Therapy

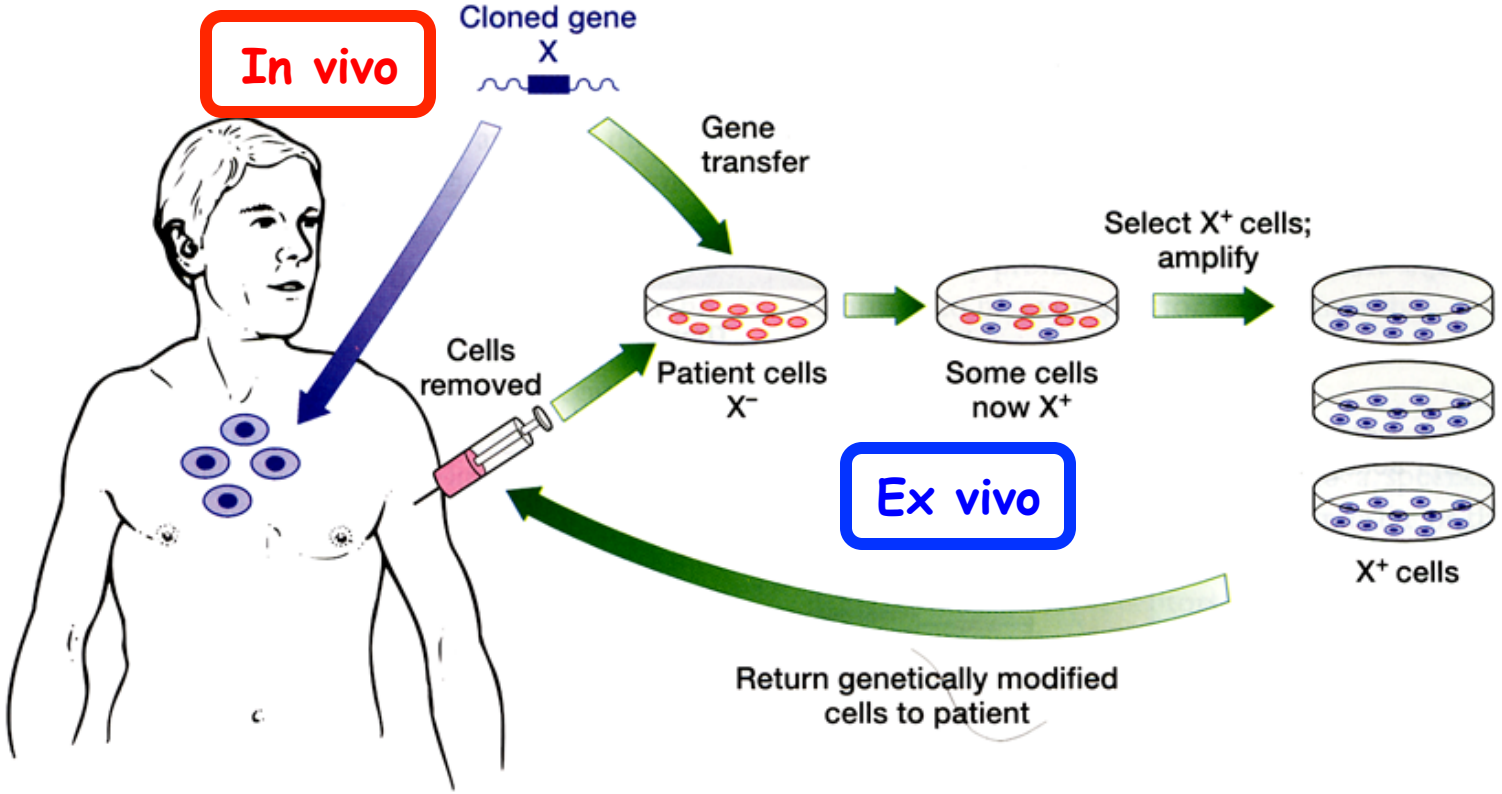
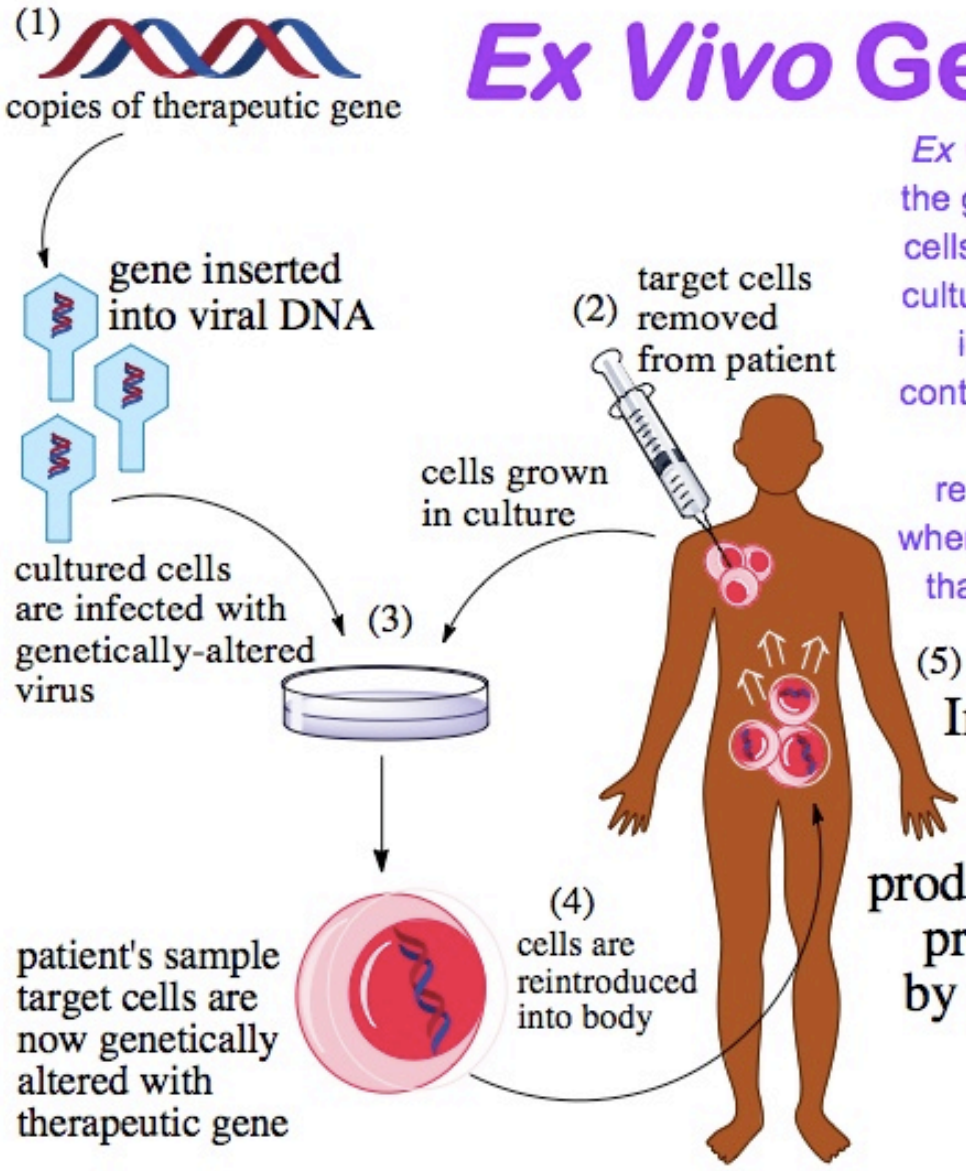


Figure 21.6: *In vivo* and *ex vivo* gene therapy.

Where possible, cells are removed from the patient, modified in the laboratory and returned to the patient (*ex vivo* gene therapy; green arrows). This allows just the appropriate cells to be treated, and the cells can be checked before they are replaced to make sure that the desired change has been achieved. For many tissues this is not possible and the cells must be modified within the patient's body (*in vivo* gene therapy; blue arrow).

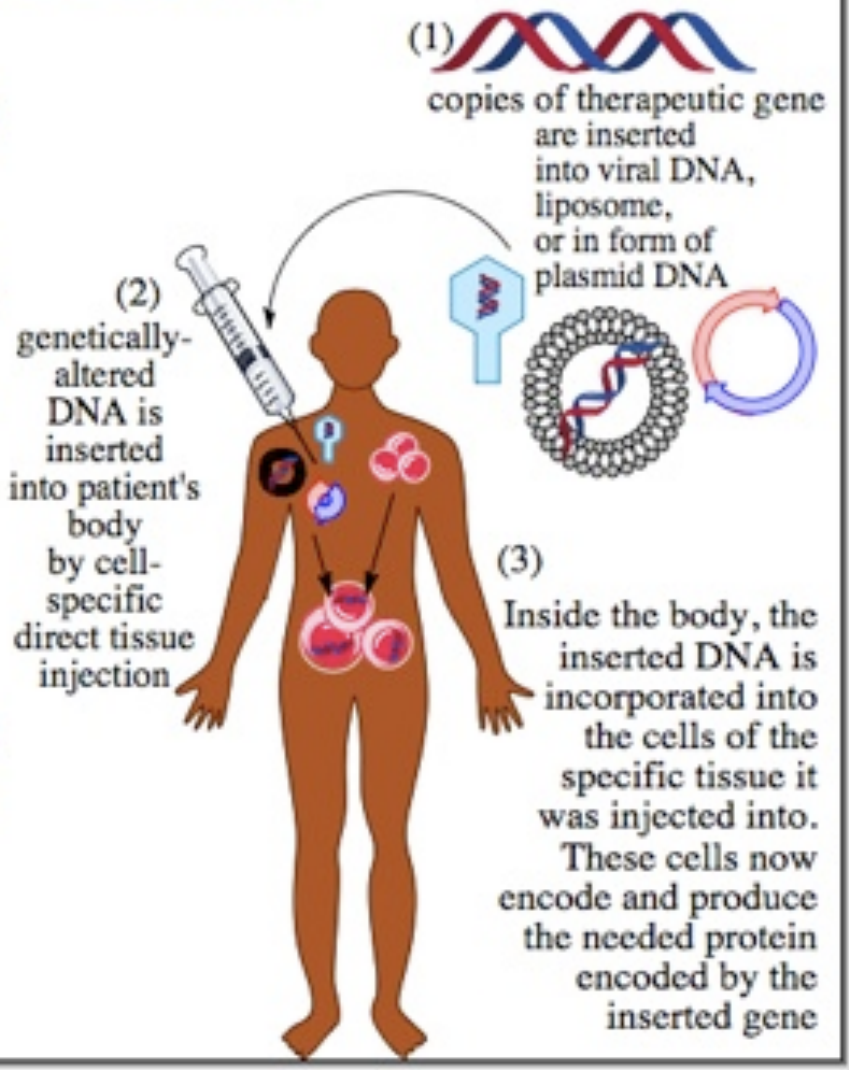
Ex Vivo Gene Therapy

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.



In Vivo Gene Therapy

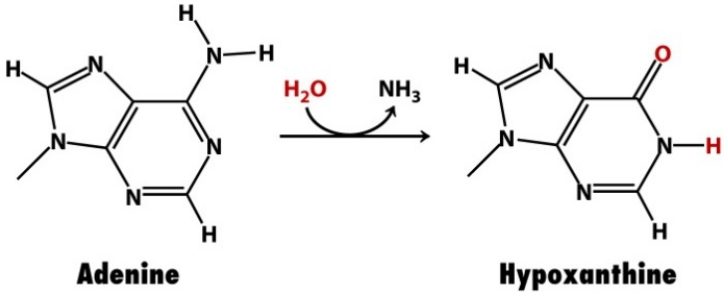
In vivo gene therapy involves introduction of therapeutic DNA directly into the patient's body. The DNA is introduced by cell-specific direct injection into tissue in need. DNA in the form of a plasmid vector is introduced by a dermal vaccination. Modified liposomes are not currently used for gene therapy, 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

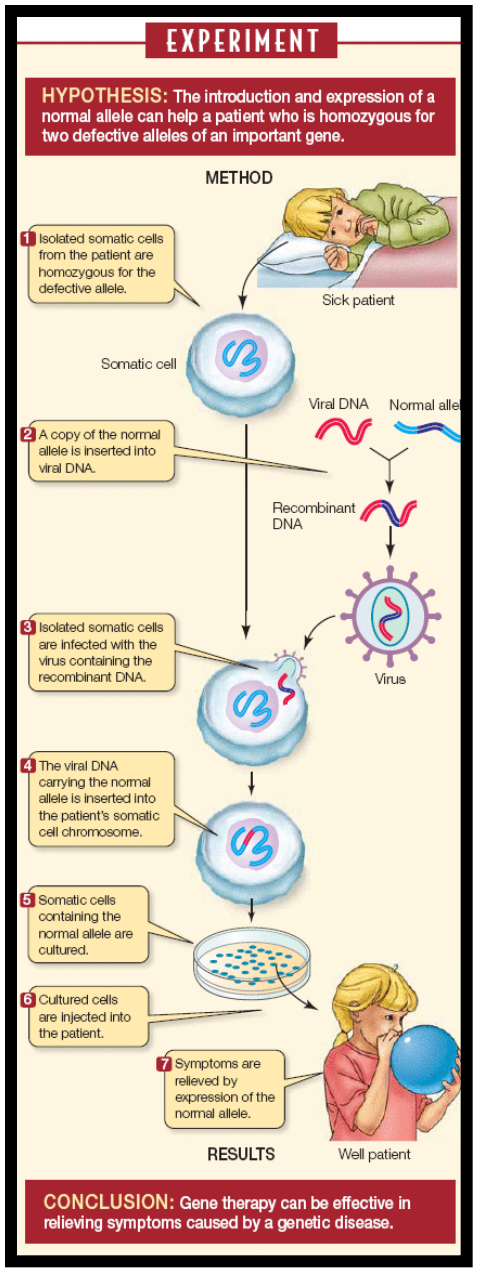


Degradation of Purine

David Vetter - Died at Age 12

- 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 Normal ADA Gene!!!

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

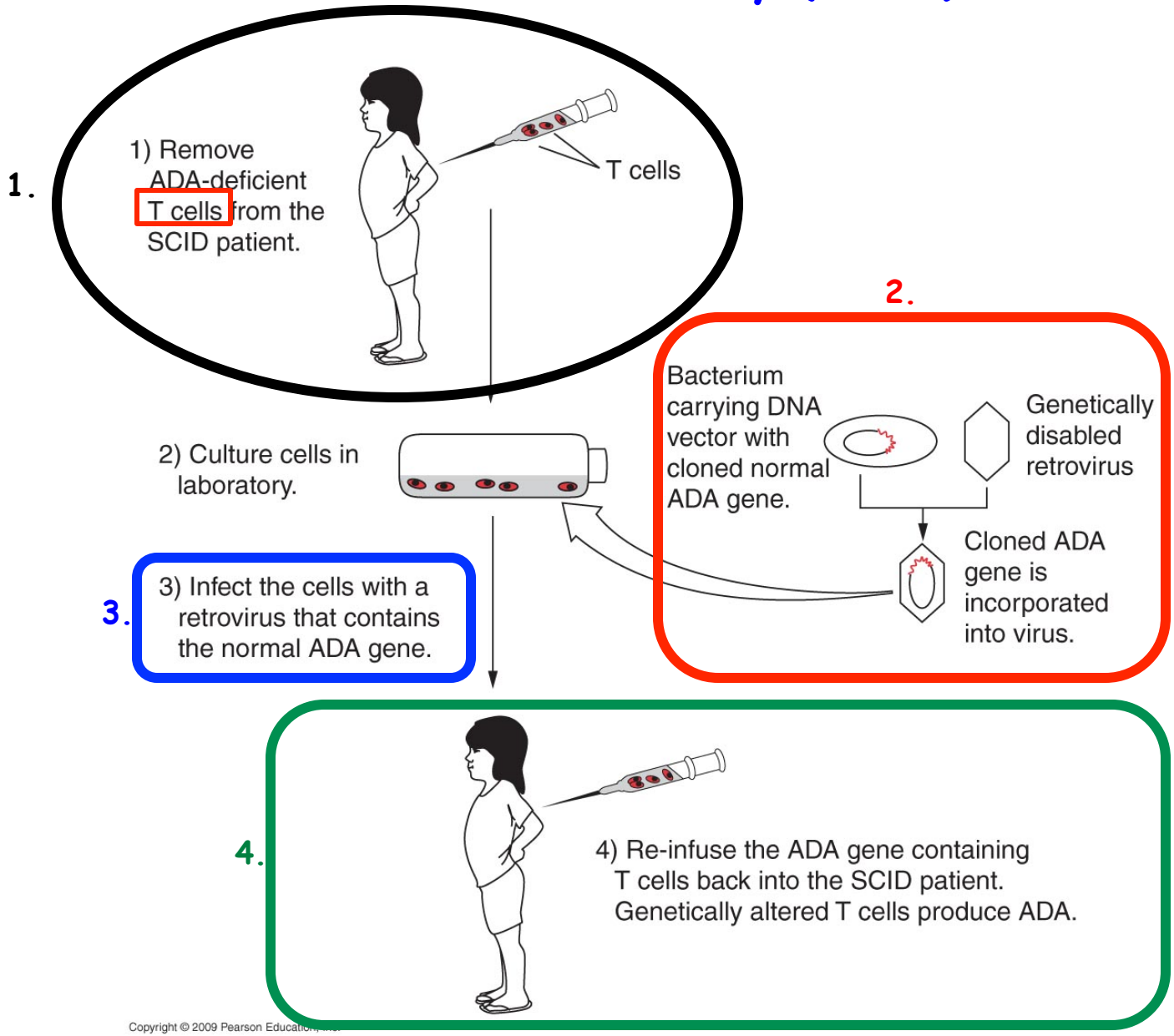
The new england journal of medicine

established in 1812 january 29, 2009 vol. 360 no. 5

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)



Vectors Used to Deliver Genes to Cells in Gene Therapy

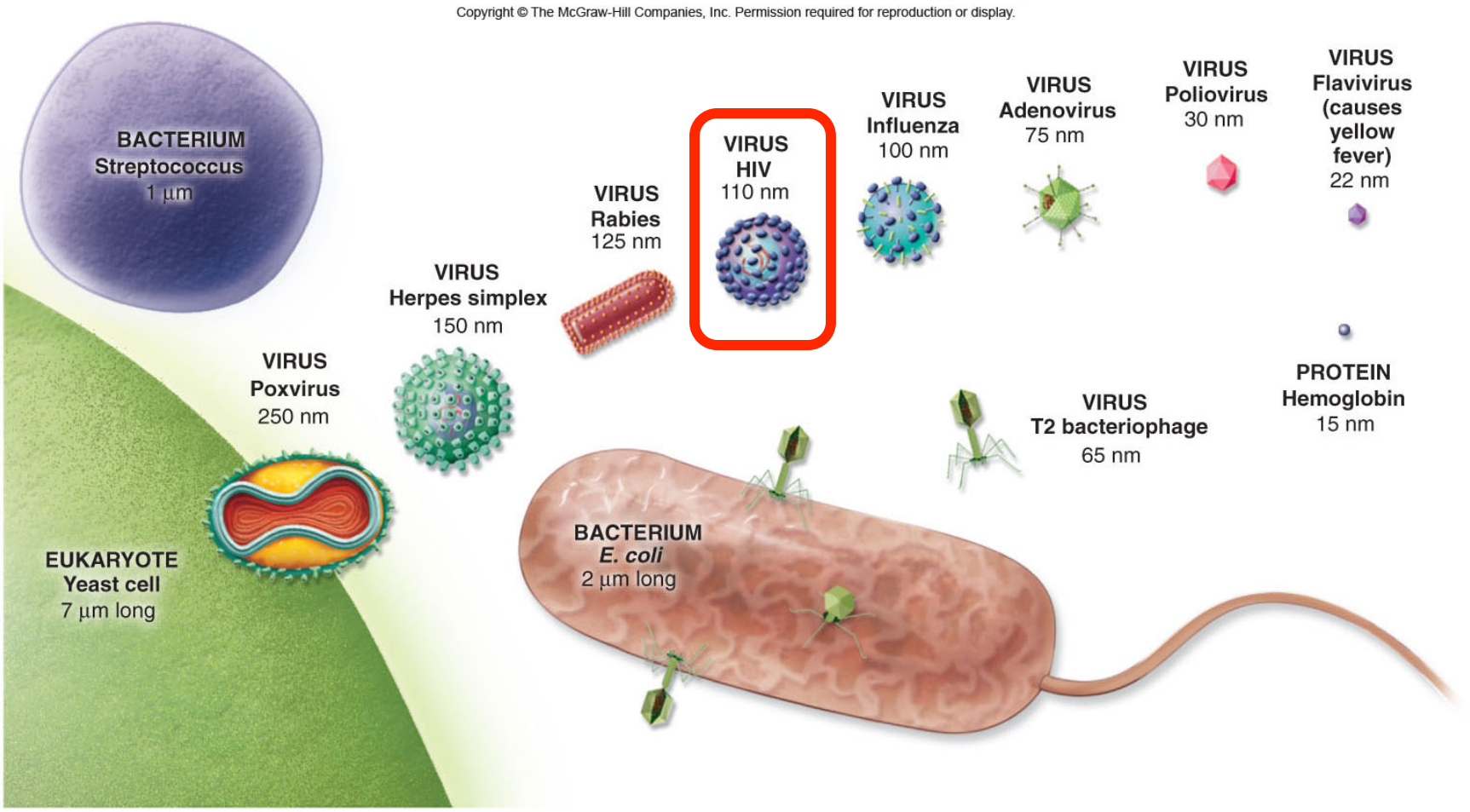
Table 19.3 Vectors used in gene therapy

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

Source: After E. Marshall, Gene therapy's growing pains, *Science* 269:1050–1055, 1995.

Table 19-3
Genetics: A Conceptual Approach, Third Edition

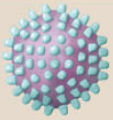
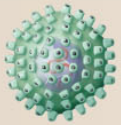
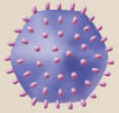
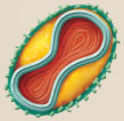
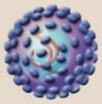
Comparison of Virus and Cell Sizes



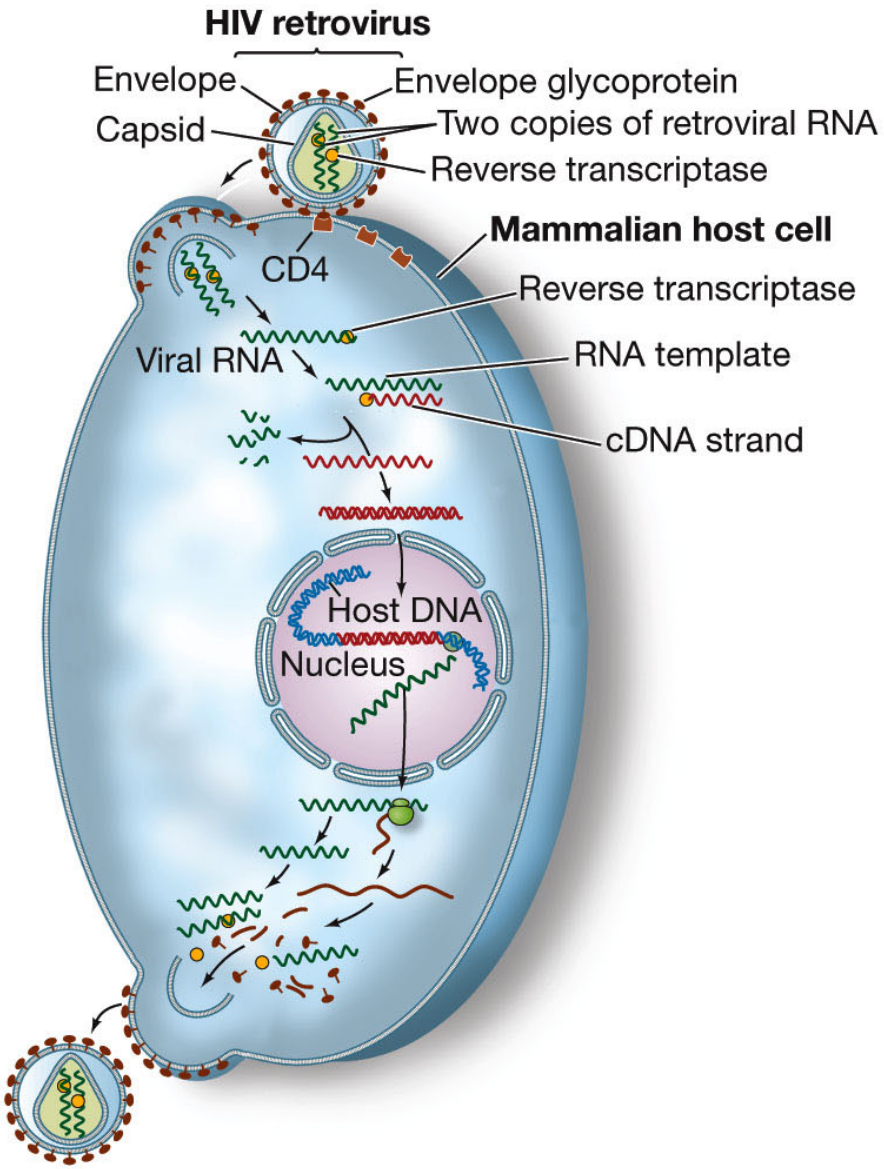
Note: 1 nm = 10^{-9} m

Human Retroviruses Are Used As Gene Therapy Vectors

TABLE 27.1 Important Human Viral Diseases

Disease	Pathogen	Genome	Vector/Epidemiology
Chicken pox	Varicella zoster 	Double-stranded DNA	Spread through contact with infected individuals. No cure. Rarely fatal. Vaccine approved in U.S. in early 1995.
Hepatitis B (viral)	Hepadnavirus 	Double-stranded DNA	Highly infectious through contact with infected body fluids. Approximately 1% of U.S. population infected. Vaccine available. No cure. Can be fatal.
Herpes	Herpes simplex virus 	Double-stranded DNA	Blisters; spread primarily through skin-to-skin contact with cold sores/blisters. Very prevalent worldwide. No cure. Exhibits latency—the disease can be dormant for several years.
Mononucleosis	Epstein–Barr virus 	Double-stranded DNA	Spread through contact with infected saliva. May last several weeks; common in young adults. No cure. Rarely fatal.
Smallpox	Variola virus 	Double-stranded DNA	Historically a major killer; the last recorded case of smallpox was in 1977. A worldwide vaccination campaign wiped out the disease completely.
AIDS	HIV 	(+) Single-stranded RNA (two copies)	Destroys immune defenses, resulting in death by infection or cancer. As of 2005, 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.
Polio	Enterovirus 	(+) Single-stranded RNA	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.

HIV is a Retrovirus



T-Cell

LIFE 8e, Figure 13.6

Discovery of Retroviruses

The Retrovirus Genome Encodes Reverse Transcriptase

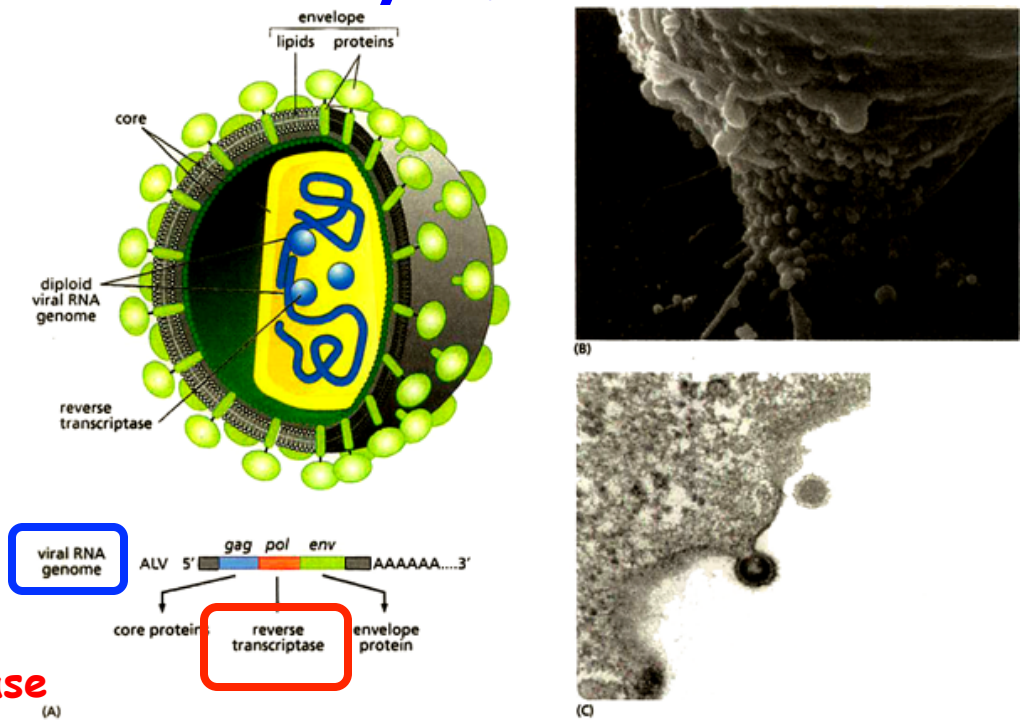


Figure 3.4 The virion of RSV and related viruses (A) This schematic drawing of the structure of a *retrovirus* virion, such as that of Rous sarcoma virus, indicates three major types of viral proteins. The glycoprotein spikes (encoded by the viral *env* gene) protrude from the lipid bilayer that surrounds the virion; these spikes enable the virion to *adsorb* (attach) to the surface of a cell and to introduce the internal contents of the virion into its cytoplasm. These include a complex protein coat formed by the several core proteins encoded by the viral *gag* gene. Within this protein shell are found two identical copies of the viral genomic the viral *pol* gene. (B) Scanning electron micrograph and (C) transmission electron micrograph showing murine leukemia virus (MLV) particles budding from the surface of an infected cell. As the nucleocapsids (containing the gag proteins, the virion RNA, and the reverse transcriptase) leave the cell, they wrap themselves with a patch of lipid bilayer taken from the plasma membrane of the infected cell. (A, adapted from H. Fan et al., The Biology of AIDS. Boston, MA: Jones and Bartlett Publishers, 1989; B, courtesy of Albert Einstein College of Medicine; C, courtesy of Laboratoire de Biologie Moleculaire.)

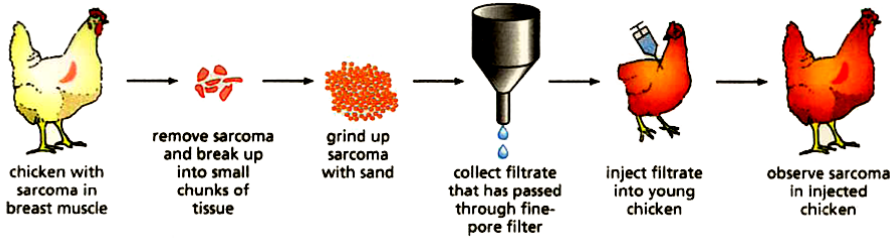
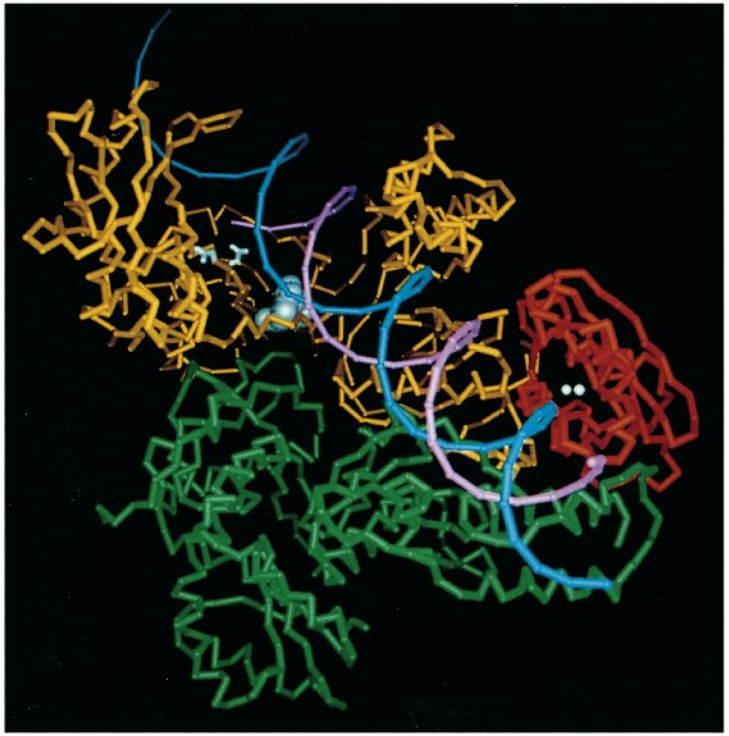


Figure 3.2 Rous's protocol for inducing sarcomas in chickens Rous removed a sarcoma from the breast muscle of a chicken, ground it with sand, and passed the resulting homogenate through a fine-pore filter. He then injected the filtrate (the liquid that passed through the filter) into the wing web of a young chicken and observed the development of a sarcoma many weeks later. He then ground up this new sarcoma and repeated the cycle of homogenization, filtration, and injection, once again observing a tumor in another young chicken. These cycles could be repeated indefinitely; after repeated serial passaging, the virus was able to produce sarcomas far more rapidly than the original viral isolate.

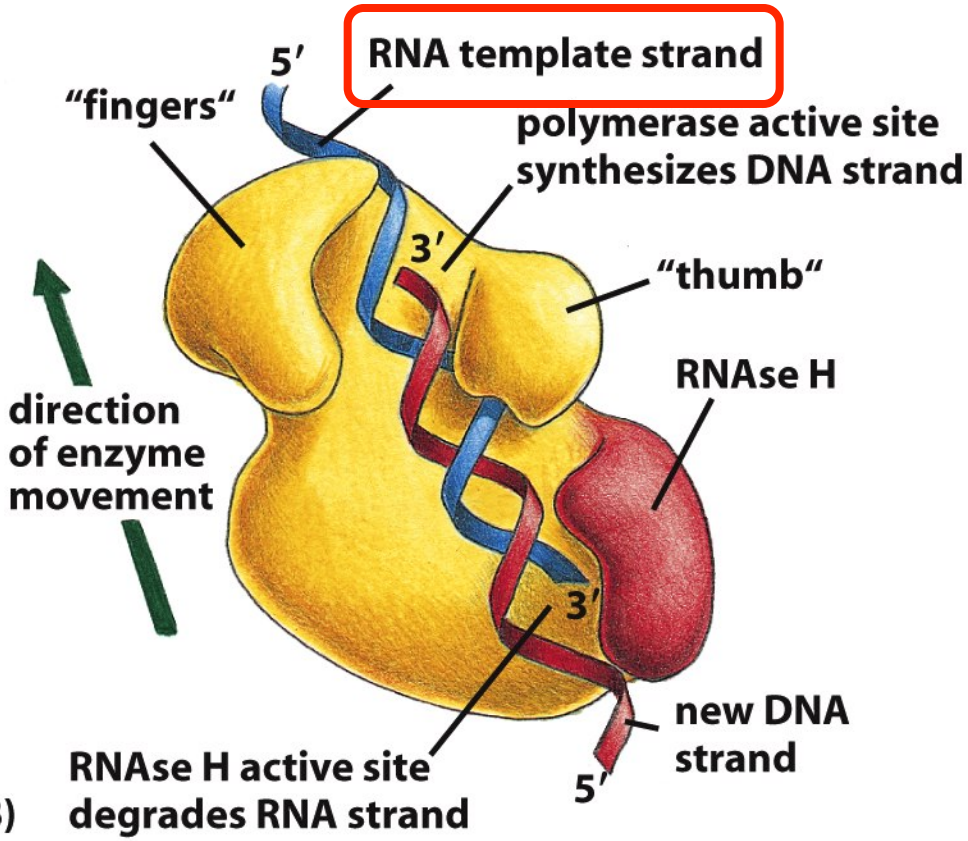
Rous Sarcoma Virus is a Retrovirus That Cases Cancer and Contains Oncogenes in its Genome

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



(A)

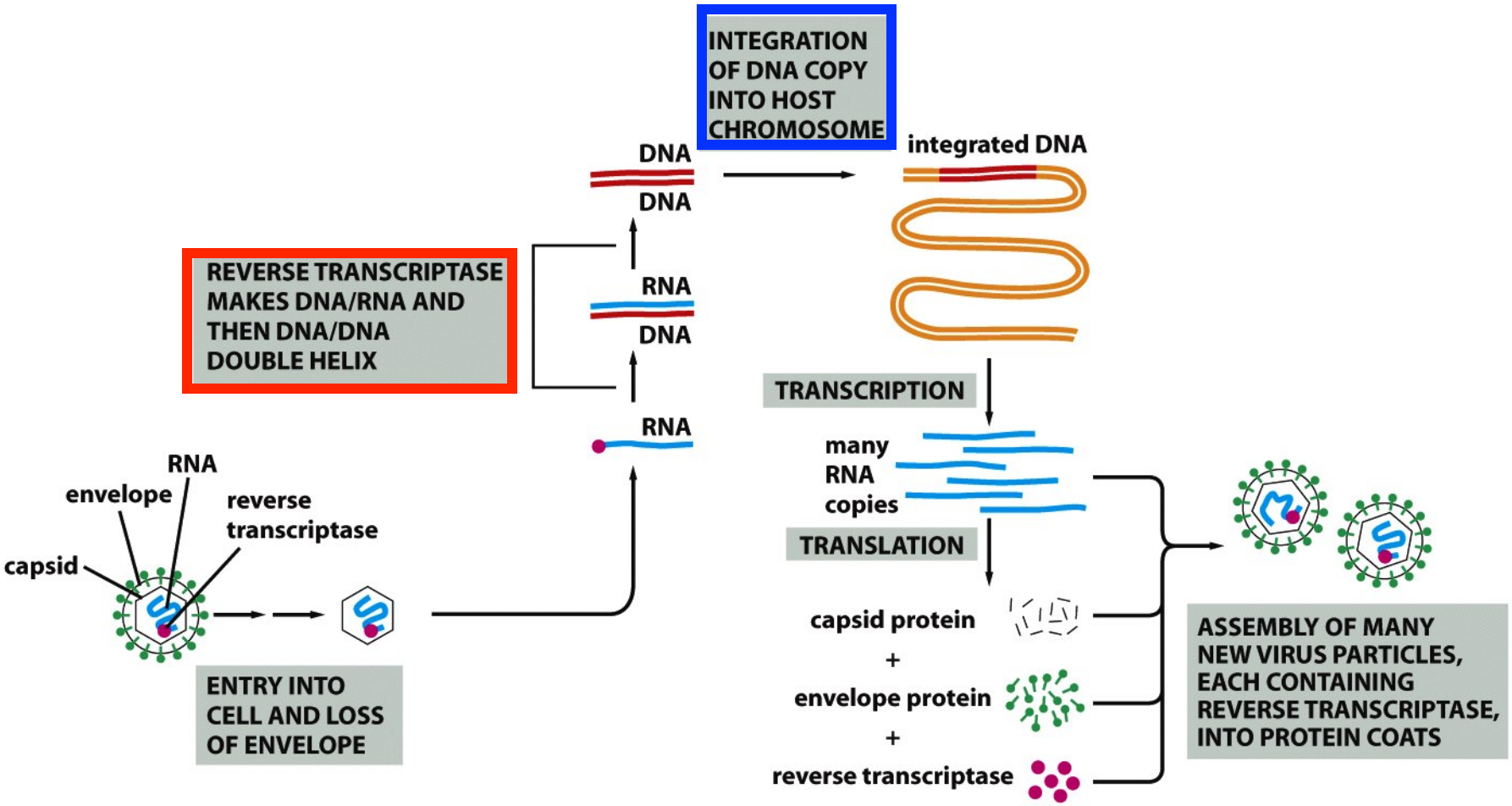


(B)

Reverse Transcriptase

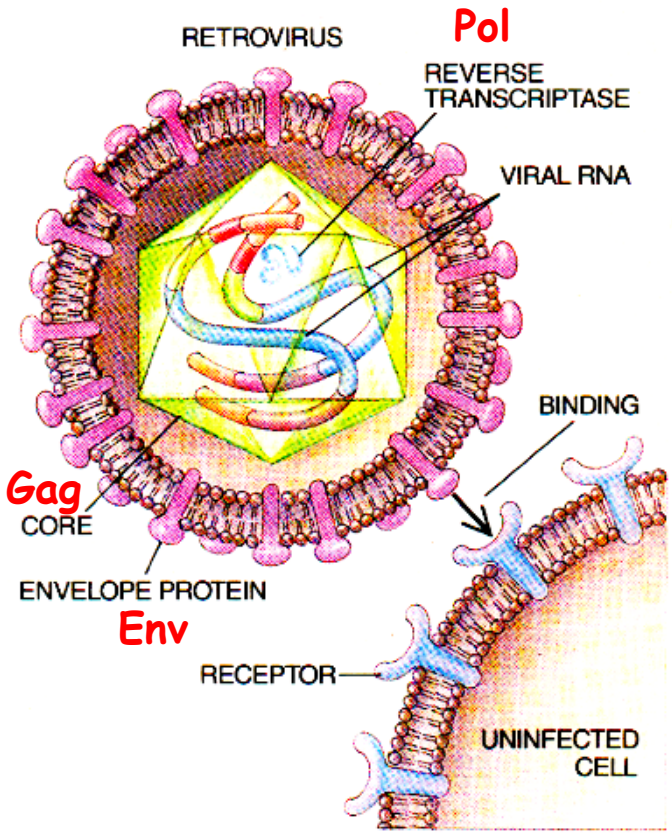
Figure 5-72 *Molecular Biology of the Cell* (© Garland Science 2008)

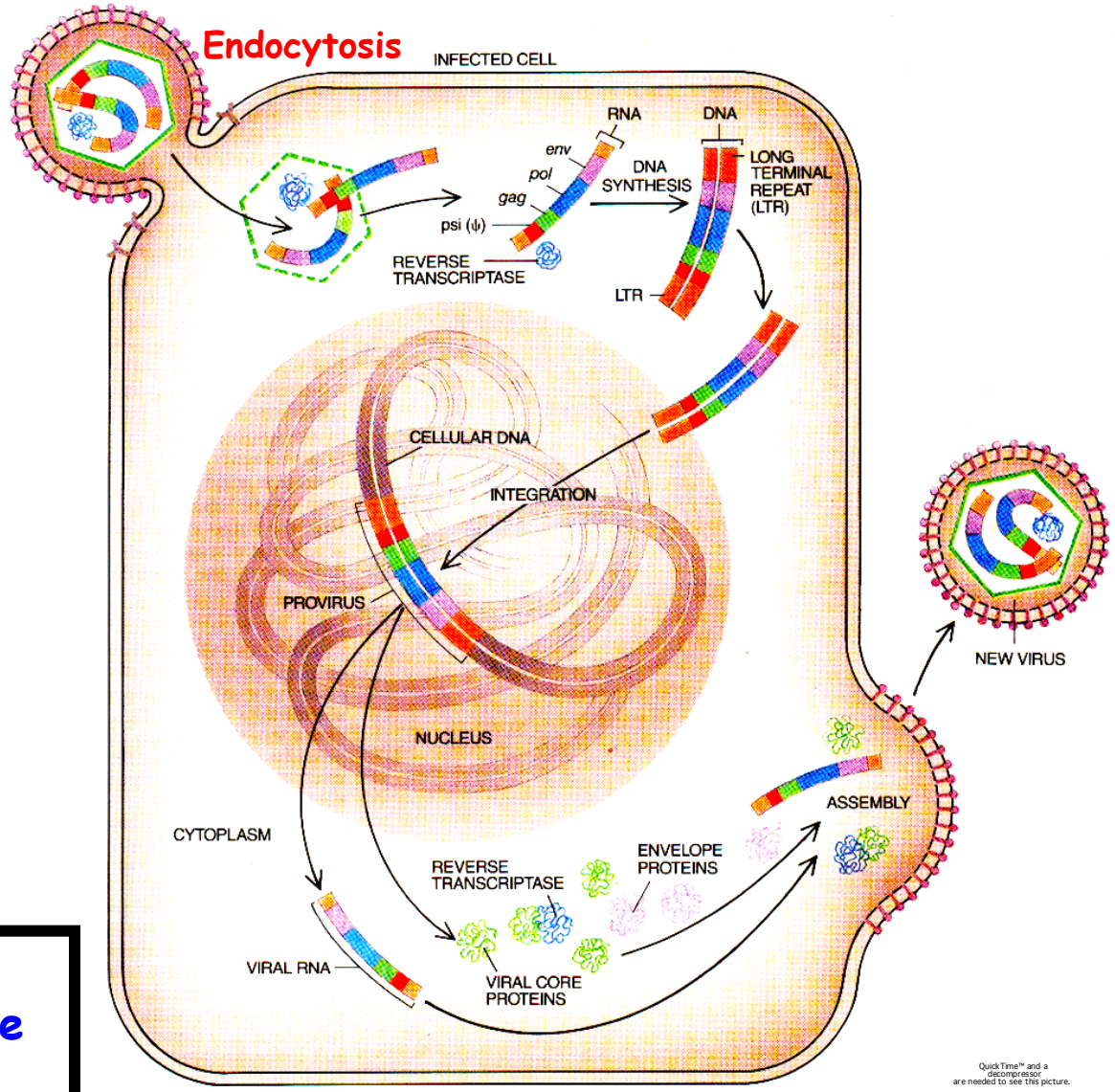
Retrovirus Life Cycle



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

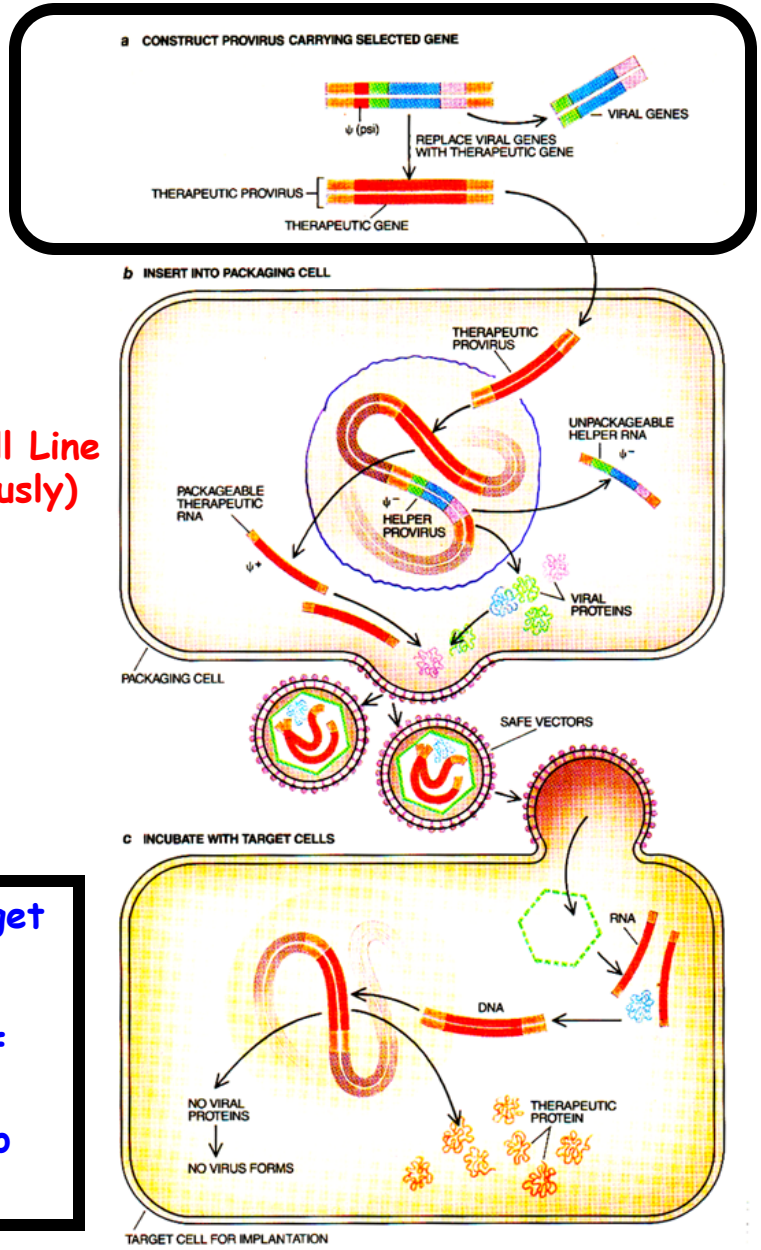
Using a Retrovirus as a Vector For Human Ex Vivo Gene Therapy





- Gag = Capid Protein
- Pol = Reverse Transcriptase
- Env = Envelope Protein
- Ψ (Psi) = Packaging Sequence

Using Retroviruses for Ex Vivo Gene Therapy



A.

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

B.

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

Packaging Cell Line (Made Previously)

C.

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?

Science
AAAS

T Lymphocyte–Directed Gene Therapy for ADA⁻ SCID: Initial Trial Results After 4 Years

R. Michael Blaese,* Kenneth W. Culver, A. Dusty Miller, Charles S. Carter, Thomas Fleisher, Mario Clerici,† 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⁻ 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

Some Problems With Human Gene Therapy

- Delivery Systems To Target Cells
- Gene Expression Levels
- 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)

Setbacks for Gene Therapy

The New York Times 1999

The Biotech Death of Jesse Gelsinger

By Sheryl Gay Stolberg
Published: November 28, 1999

- Had a mild form of ornithine transcarbamylase deficiency – results in an inability to metabolize ammonia
- Volunteered for clinical trial of gene supplementation therapy and was injected with adenovirus vector containing OTC gene
- Died of systemic inflammatory response syndrome – immune reaction to adenovirus vector

BBC NEWS 2003

Gene therapy 'caused leukaemia'

- 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

A Comeback for Gene Therapy

The new england journal of medicine
established in 1812 january 29, 2009 vol. 360 no. 5

Gene Therapy for Immunodeficiency Due to Adenosine Deaminase Deficiency

November 3, 2009

Giving Sight by Therapy With Genes

Gene Therapy for Metastatic Melanoma in Mice Produces Complete Remission

ScienceDaily (Nov. 18, 2010) — A potent anti-tumor gene introduced into mice with metastatic melanoma has resulted in permanent immune reconfiguration and produced a complete remission of their cancer, according to an article to be published in the December 2010 issue of the *Journal of Clinical Investigation*.

nature Vol 461|8 October 2009|doi:10.1038/nature08401

LETTERS

Gene therapy for red–green colour blindness in adult primates

Katherine Mancuso[1], William W. Hauswirth[2], Qiuhong Li[2], Thomas B. Connor[3], James A. Kuchenbecker[1], Matthew C. Mauck[3], Jay Neitz[1] & Maureen Neitz[1]

Science AAAS
A Comeback for Gene Therapy
Luigi Naldini
Science **326**, 805 (2009);
DOI: 10.1126/science.1181937

New Anti-HIV Gene Therapy Makes T-Cells Resistant to HIV Infection

ScienceDaily (Jan. 26, 2011) — An innovative genetic strategy for rendering T-cells resistant to HIV infection without affecting their normal growth and activity is described in a paper published in *Human Gene Therapy*, a peer-reviewed journal published by Mary Ann Liebert, Inc.

Vol 467|16 September 2010 nature

NEWS & VIEWS

GENE THERAPY

Targeting β-thalassaemia

Derek A. Persons

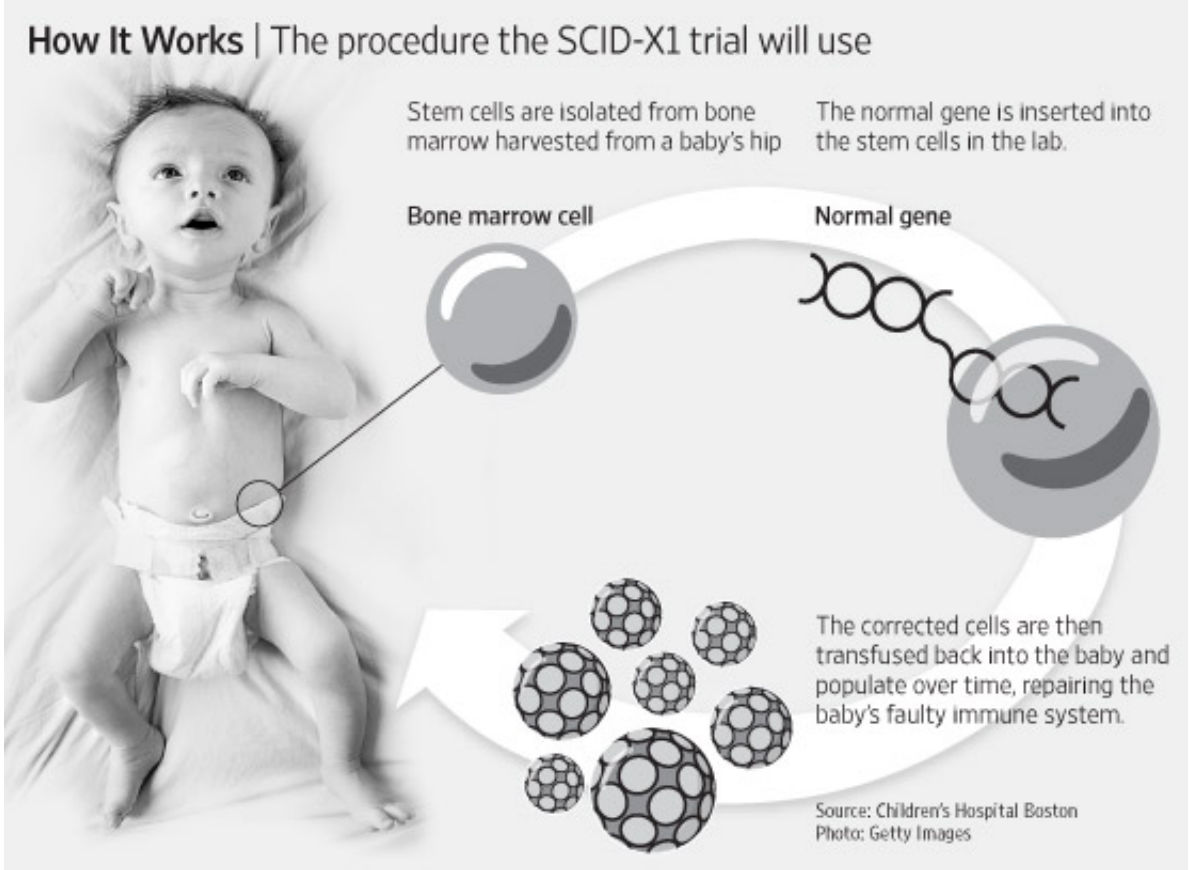
Patients with disorders of the blood protein haemoglobin often depend on lifelong blood transfusions. That could change, given the success of gene therapy in a patient with one such disorder.

Virus-Based Gene Therapy for Metastatic Kidney Cancer Developed

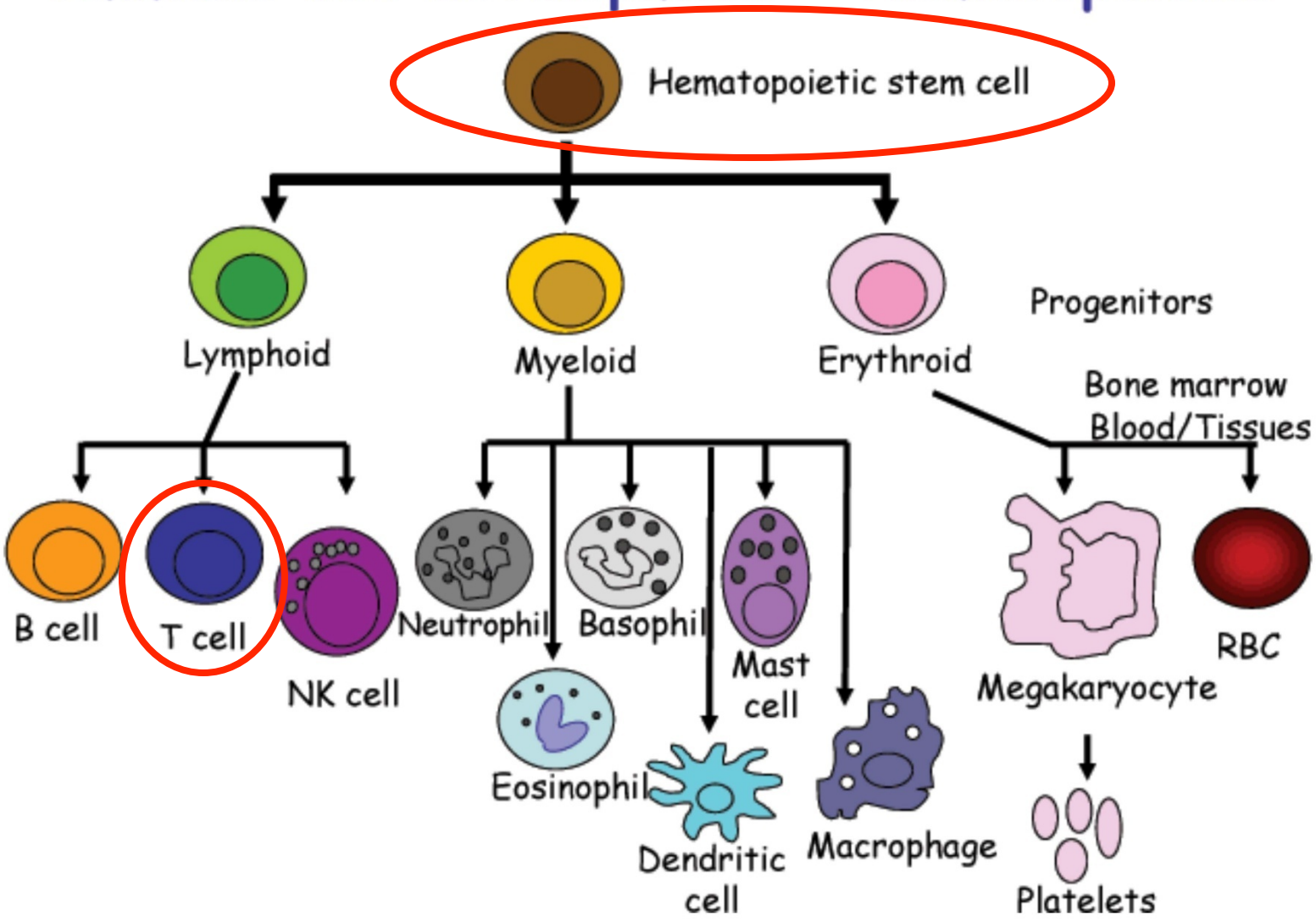
ScienceDaily (Dec. 19, 2010) — Researchers at Virginia Commonwealth University Massey Cancer Center and the VCU Institute of Molecular Medicine (VIMM) have developed a novel virus-based gene therapy for renal cell carcinoma that has been shown to kill cancer cells not only at the primary tumor site but also in distant tumors not directly infected by the virus. Renal cell carcinoma is the most common form of kidney cancer in adults and currently there is no effective treatment for the disease once it has spread outside of the kidney.

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



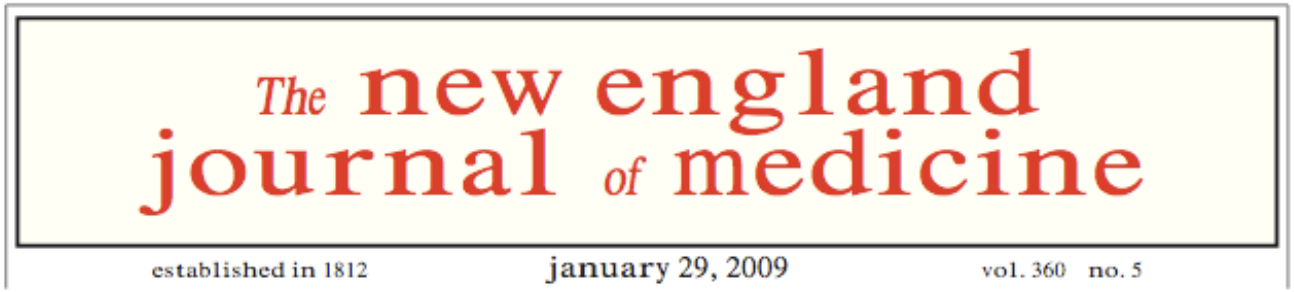
Immune cell development: Hematopoiesis



It Works!

Gene therapy cures 'bubble boy disease'

31 Jan 2009, 1128 hrs IST, AP



Gene Therapy for Immunodeficiency Due to Adenosine Deaminase Deficiency

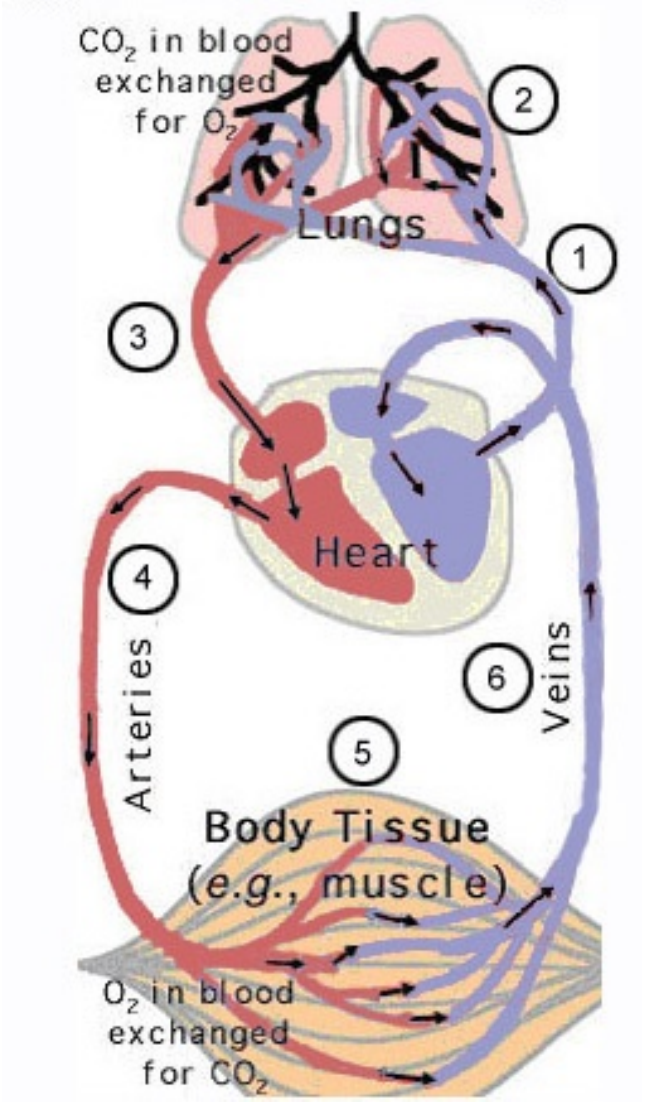
Results after 10 years

- 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

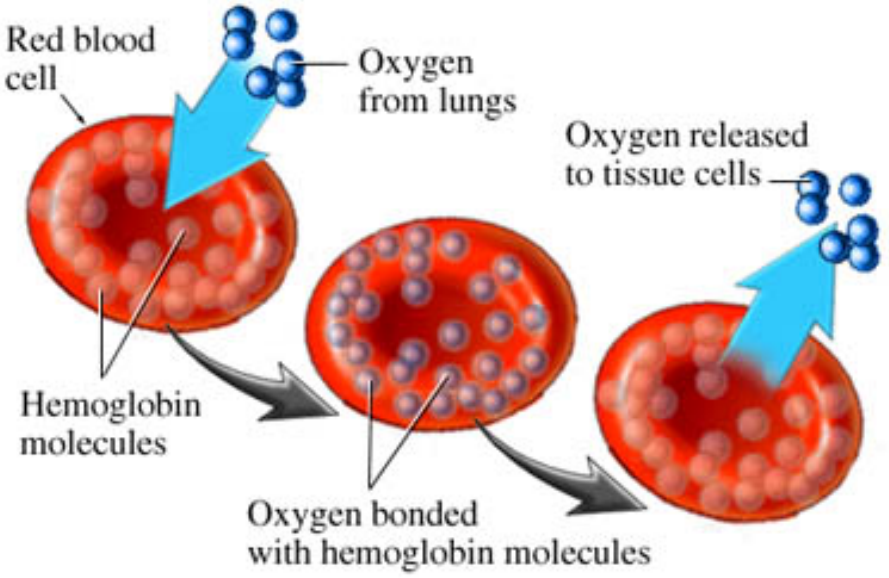
Another Example of Ex Vivo Gene Therapy Using Hemotopoietic Stem Cells

Hemoglobin & Blood Oxygen Transport

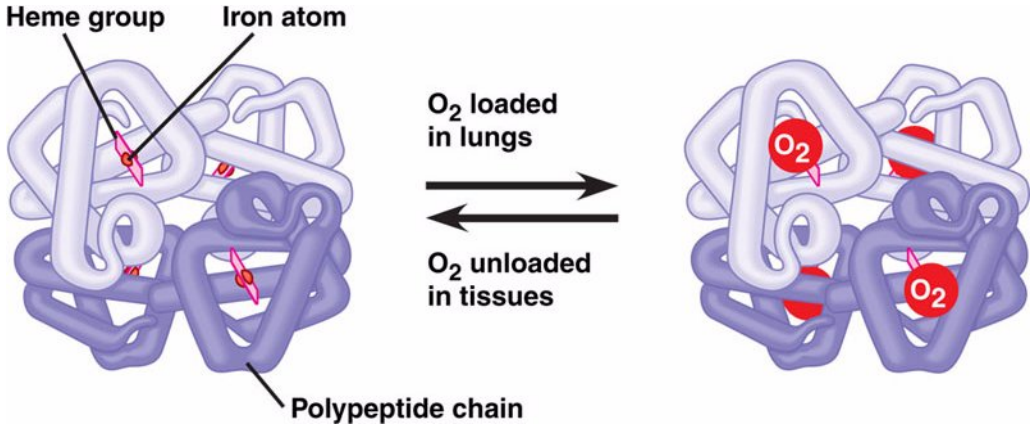
Circulatory System



Red Blood Cells and Oxygen Transport



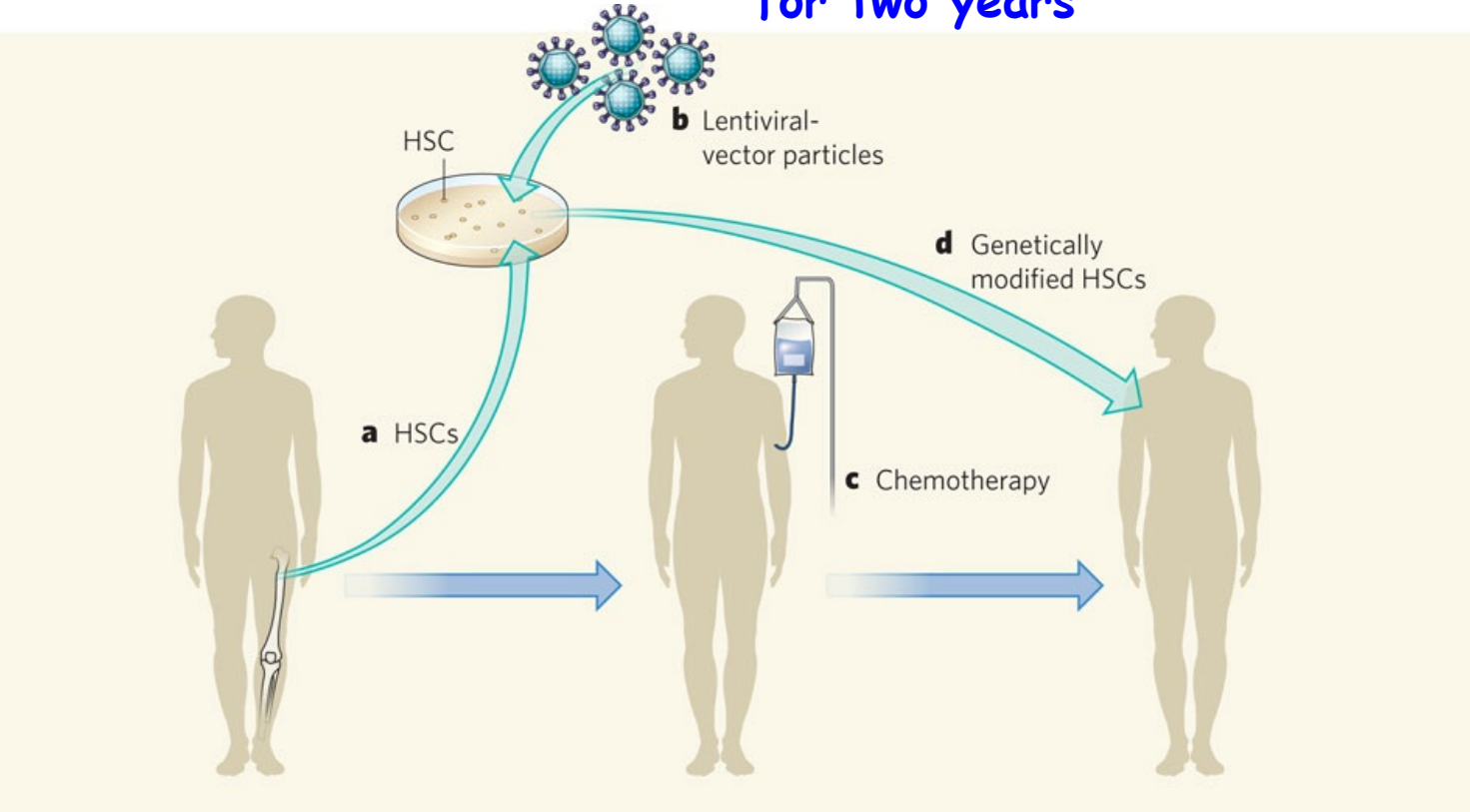
Hemoglobin Binds Oxygen



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

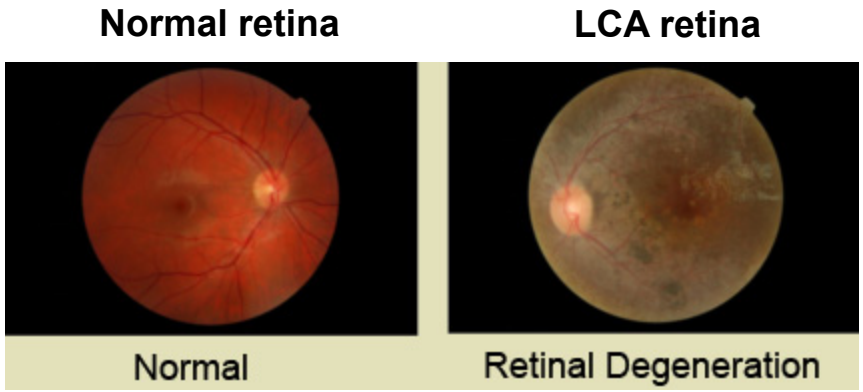
- Gene therapy – transduced hematopoietic stem cells (HSC) with lentivirus (HIV) engineered with β-globin gene
- Transplanted therapeutic HSCs into patient following chemotherapy to destroy diseased HSCs
- Patient has not needed transfusions for two years



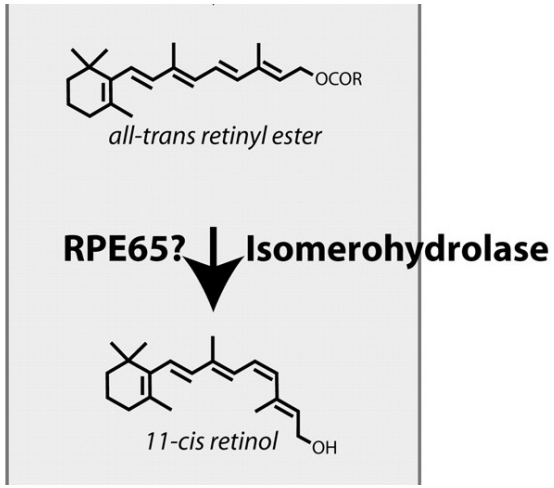
In Vivo
Gene Therapy

Blindness - Leber Congenital Amaurosis (LCA)

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

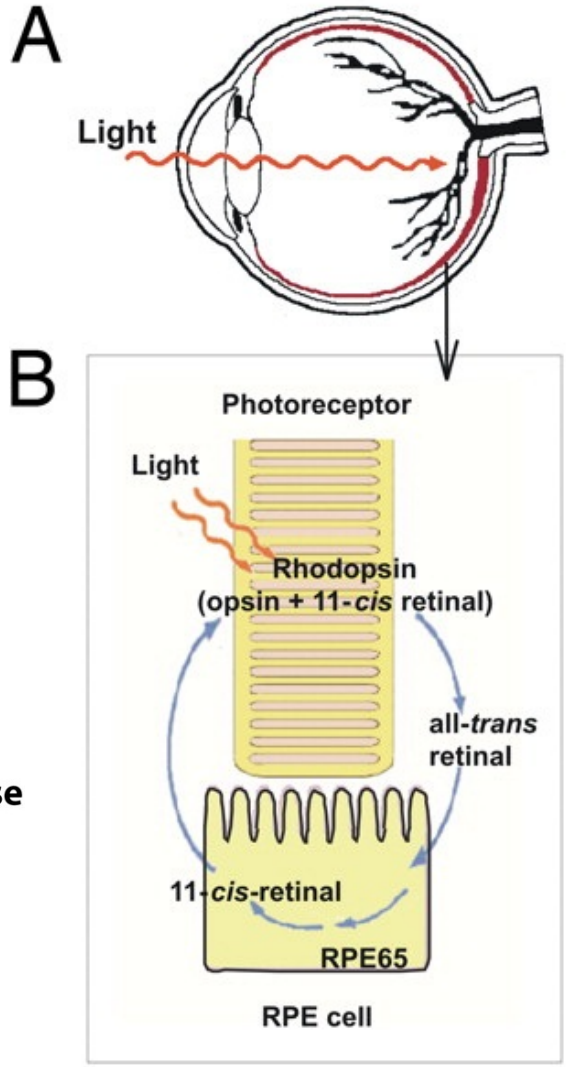


Type 2 LCA is caused by recessive mutations in the RPE65 isomerase gene



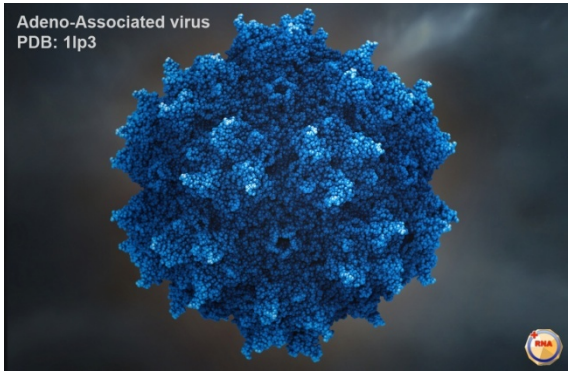
Moiseyev G et al. PNAS 2005;102:12413-12418

How We See



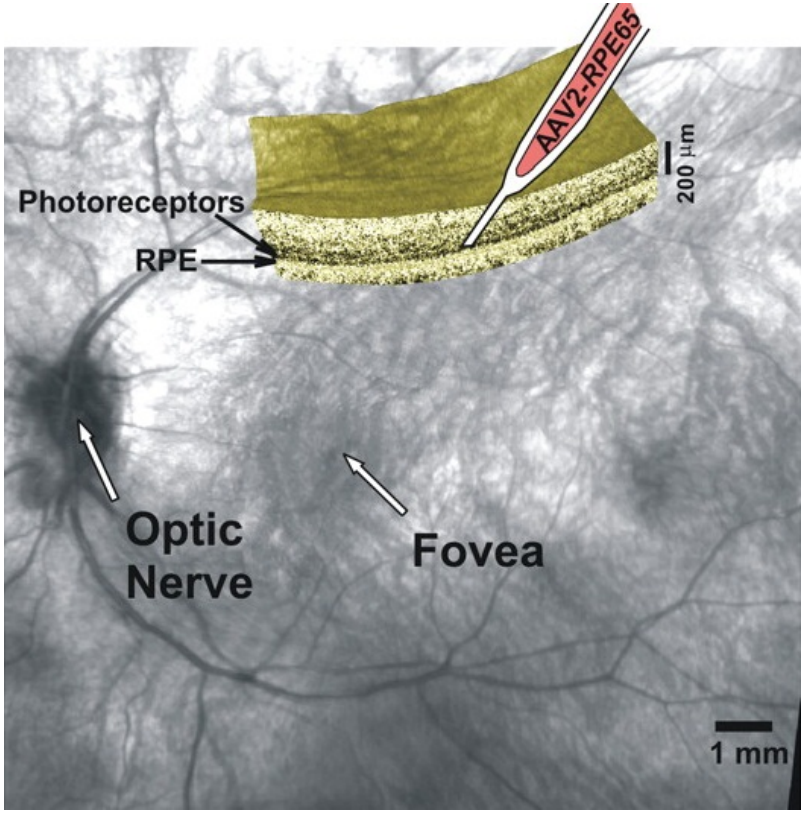
Cideciyan et al. PNAS 2008;105:15112

LCA Gene Therapy Using RPE65 & AAV2



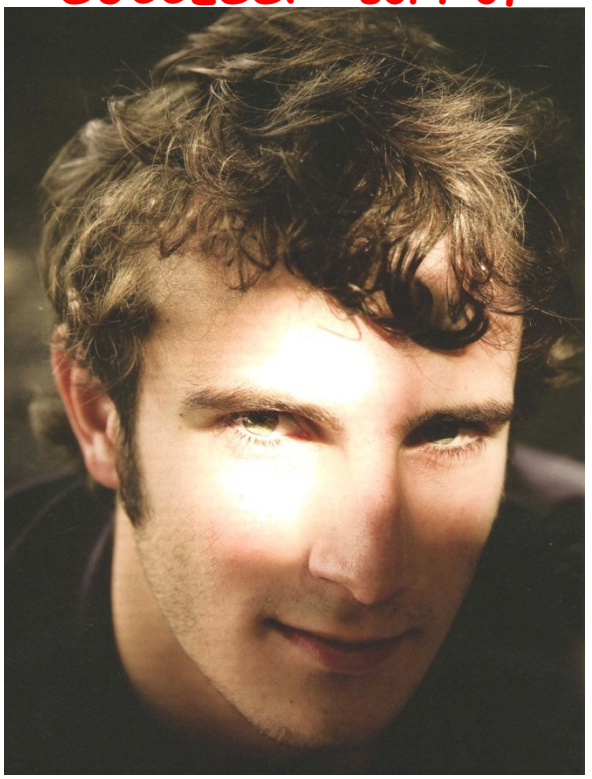
Adeno-associated viruses (AAV)

- Does not generally provoke antibody formation
- Infects nondividing cells of many different tissues
- Little or no integration of viral DNA into the host genome
- Has a small genome and can carry only short segments of DNA



Cideciyan et al. PNAS 2008;105:15112

SUCCESS! – sort of



ALESSANDRO CANNATA

Are Two Eyes Better than One?

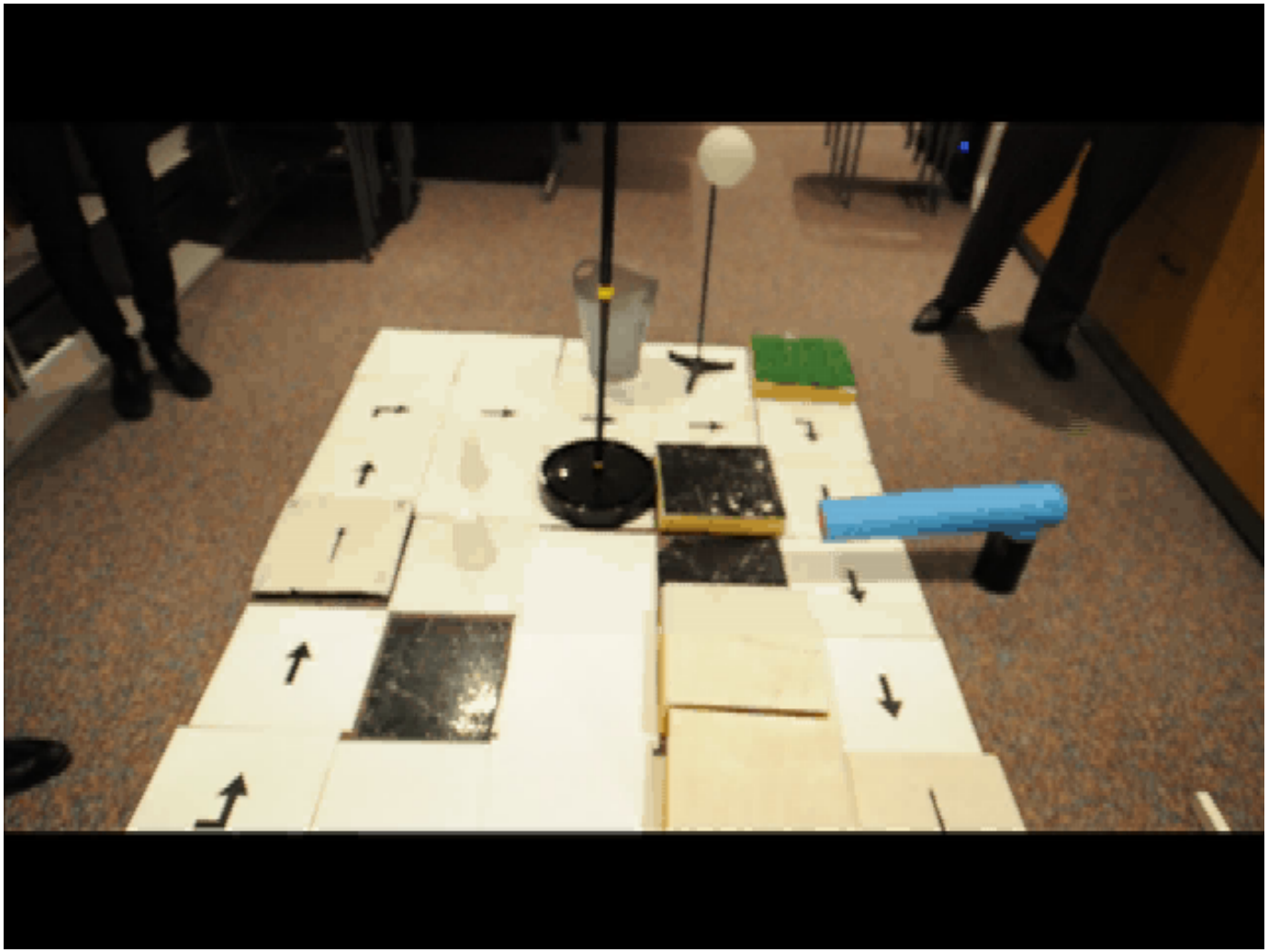
- **Question**
  - Can the second eye of LCA patients who had previously undergone RPE65 gene therapy be treated?
- **Protocol**
  - RPE65 gene administered with the AAV2 vector to 3 of the original 12 patients 1.7 to 3.3 years after initial treatment
- **Results**
  - Second treatment was safe and effective.

Science Translational Medicine AAAS

AAV2 Gene Therapy Readministration in Three Adults with Congenital Blindness
Jean Bennett, *et al.*
Sci Transl Med **4**, 120ra15 (2012);
DOI: 10.1126/scitranslmed.3002865

Are Two Eyes Better than One?

Before



Are Two Eyes Better than One?

After

Target for in Vivo Gene Therapy: Hemophilia B that is Caused by Mutations in Factor IX Gene

TABLE 13.2 Some Important Genetic Disorders

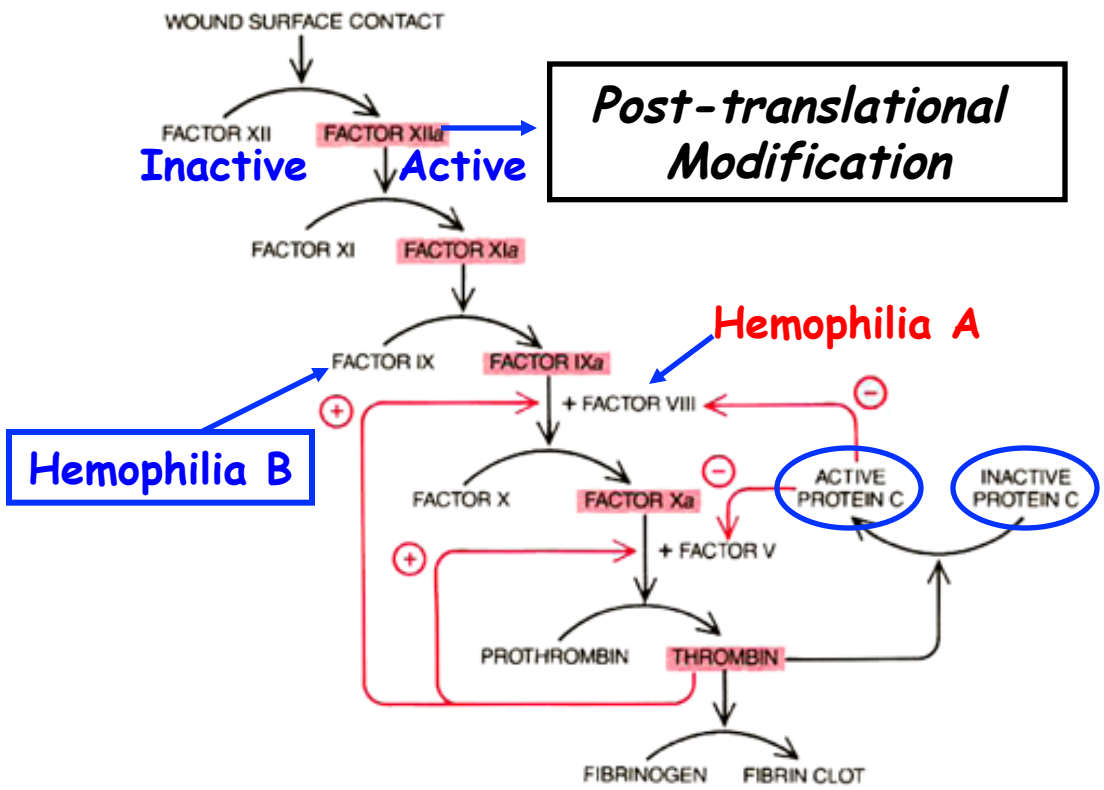
Disorder	Symptom	Defect	Dominant/ Recessive	Frequency Among Human Births
Cystic fibrosis	Mucus clogs lungs, liver, and pancreas	Failure of chloride ion transport mechanism	Recessive	1/2500 (Caucasians)
Sickle cell anemia	Blood circulation is poor	Abnormal hemoglobin molecules	Recessive	1/600 (African Americans)
Tay–Sachs disease	Central nervous system deteriorates in infancy	Defective enzyme (hexosaminidase A)	Recessive	1/3500 (Ashkenazi Jews)
Phenylketonuria	Brain fails to develop in infancy	Defective enzyme (phenylalanine hydroxylase)	Recessive	1/12,000
Hemophilia	Blood fails to clot	Defective blood-clotting factor VIII	X-linked recessive	1/10,000 (Caucasian males)
Huntington disease	Brain tissue gradually deteriorates in middle age	Production of an inhibitor of brain cell metabolism	Dominant	1/24,000
Muscular dystrophy (Duchenne)	Muscles waste away	Degradation of myelin coating of nerves stimulating muscles	X-linked recessive	1/3700 (males)
Hypercholesterolemia	Excessive cholesterol levels in blood lead to heart disease	Abnormal form of cholesterol cell surface receptor	Dominant	1/500

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

Hemophilia A	Defective Factor VIII Gene	1/10,000 males	80%
Hemophilia B	Defective Factor IX Gene	1/30,000 males	20%
Henophilia C	Defective Factor XI Gene	Autosomal	<1%

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

How Does Blood Clot After Wounding?



CLOTTING CASCADE begins when cell damage at a wound somehow activates the enzyme factor XII; it ends with the conversion of fibrinogen into fibrin by thrombin. At each step an inactive protein is converted into a protease, or protein-cutting enzyme (*color*), which activates the next protein. Some steps require cofactors such as factors VIII and V. The cascade includes positive- and negative-feedback loops (*colored arrows*). Thrombin activates factors VIII and V; it also deactivates them (by activating protein *C*), which helps to halt clotting. Some 85 percent of hemophiliacs lack factor VIII. The rest lack factor IX.

Post-translational Modification

Hemophilia A

Hemophilia B

Eight Proteins/Genes Required:

1. Factor VII
2. Factor XI
3. Factor IX
4. Factor VIII
5. Factor X
6. Protein C
7. Prothrombin
8. Fibrinogen

What Happens If Any Of These Proteins Or Genes Are Mutated?

↓

No Blood Clot!

Anti-Thrombin?? → **Anti-Thrombin Deficiency (At-III) genetic disease**

Cascade

December 10, 2011

The New York Times

Treatment for Blood Disease Is Gene Therapy Landmark

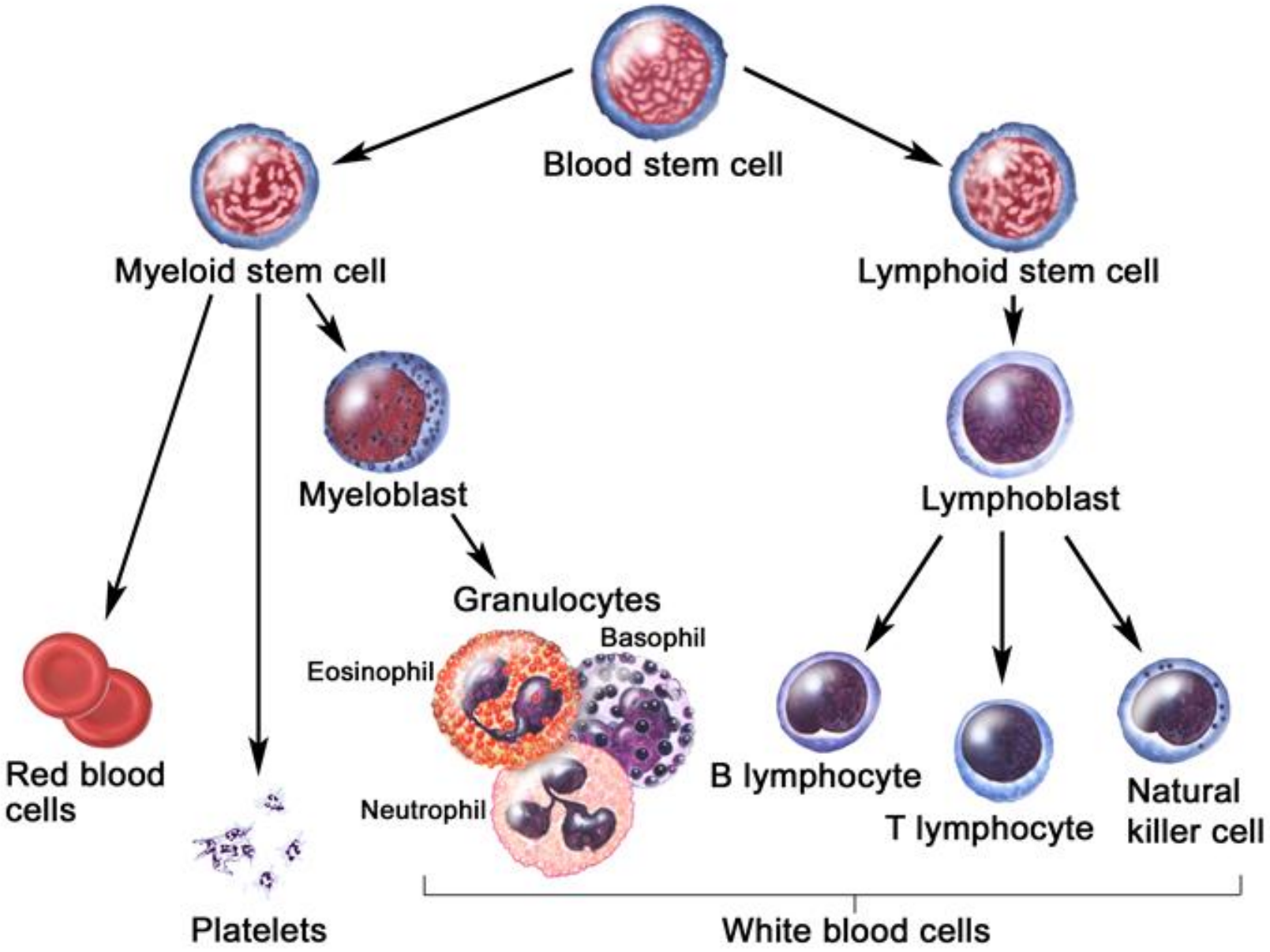
2011

- **Protocol**
  - Transferred Human Factor IX gene into adenovirus-associated virus vector that targets liver cells
  - Infused AAV8 vector into six participant 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

Adenovirus-Associated Virus Vector–Mediated Gene Transfer in Hemophilia B

Targeted Killing of Specific Cell Types

Normal Blood Cell Development



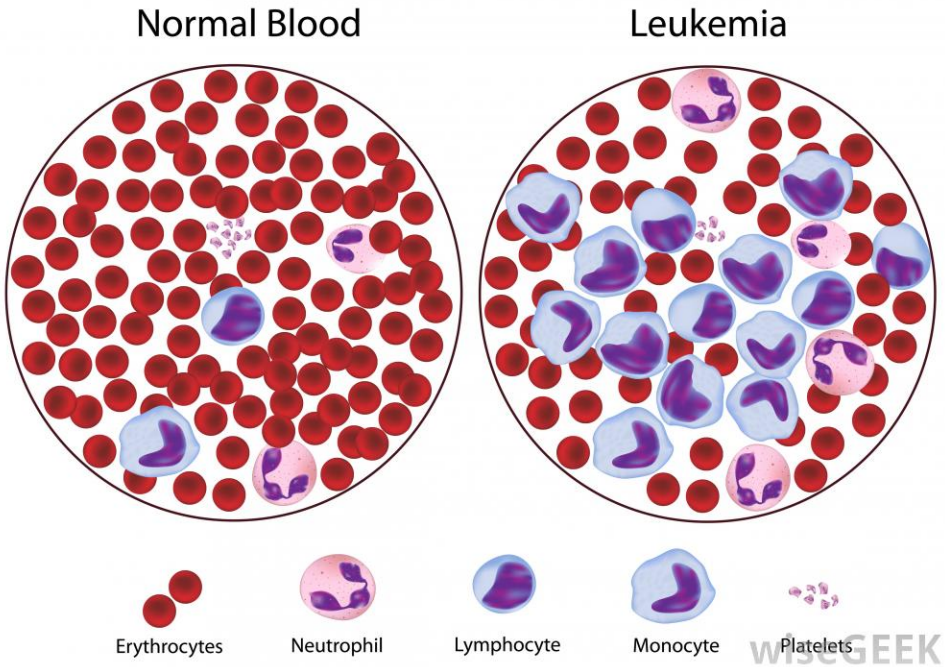
The New York Times

December 9, 2012

In Girl's Last Hope, Altered Immune Cells Beat Leukemia

By DENISE GRADY

Leukemia is cancer of the blood, that results in an increase in immature white blood cells. Chronic lymphoid leukemia affects B cell lymphocytes

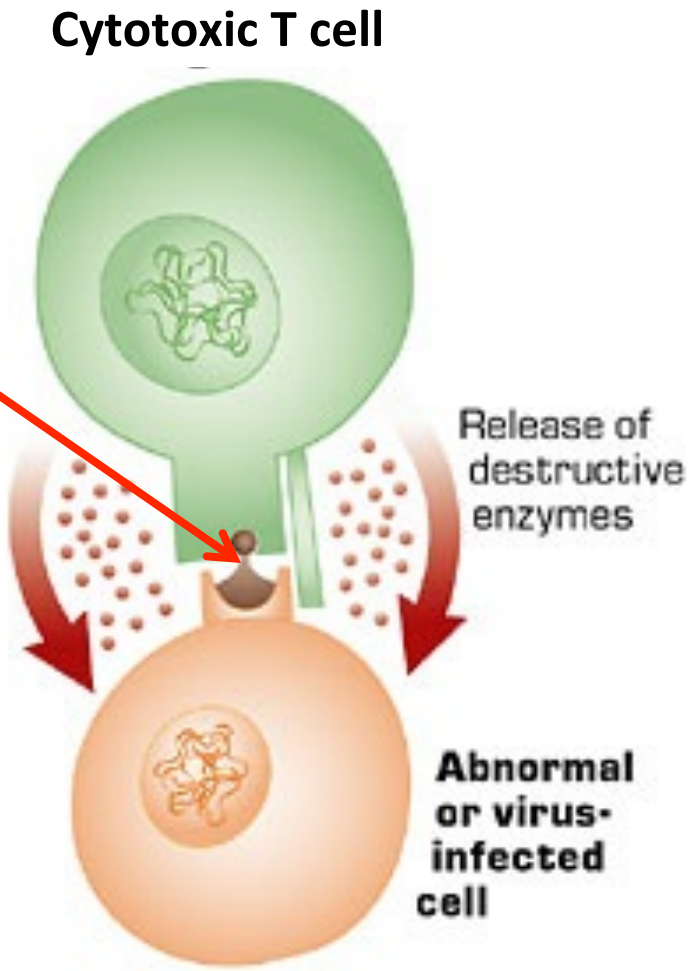


Emily Whitehead, 7, was the first child to receive gene therapy for leukemia at CHOP. (Photo courtesy of The Children's Hospital of Philadelphia)

Emily Whitehead, alive at age 7 because of a novel gene therapy strategy

Ex-vivo Gene Therapy for Chronic 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
  - 4 of 10 patients had complete remission of leukemia, and 4 improved



Science Translational Medicine AAAS

2011

Approved Gene Therapy Trials

Table 1 Selected gene therapy clinical trials

Company (location)	Therapy	Indication	Phase of development
Retrovirus			
San Raffaele	ADA-SCID GT: CD34+ cells transduced with Moloney murine leukemia virus carrying ADA gene	Primary immunodeficiencies	Phase 1/2
Ribozyme (Boulder, CO, USA)	CD34+ cells transduced with retrovirus vector with multiple ribozymes	Non-Hodgkin's lymphoma, HIV/AIDS	Phase 2
Tocagen (San Diego)	Toca-511: replication-competent retrovirus with prodrug activator cytosine deamidase gene injected into tumor	Glioma	Phase 1/2
Lentivirus			
Bluebird Bio	LentiGlobin: introduces globin gene into patient hematopoietic stem cells	β-thalassemia and sickle cell anemia	Phase 1/2
Lentigen	LG-740: T cells treated *ex vivo* with lentivirus with chimeric T-cell receptor gene	B-cell leukemia and lymphoma	Phase 1
Oxford BioMedica	ProSavin: lentivirus with three genes required for dopamine biosynthesis injected into striatum of brain	Parkinson's disease	Phase 1/2
Adenovirus			
Advantagene (Auburndale, MA, USA)	ADV-tk: replication-deficient adenovirus with herpes simplex thymidine kinase gene injected into tumor during biopsy	Glioma Pancreatic cancer	Phase 1 Phase 1
Aventis (Paris)	Ad5CMV-p53	Head and neck cancer	Phase 2
Biogen	Adenoviral mediated interferon-β	Pleural mesothelioma Colon cancer, glioma	Phase 1 Phase 1/2
GenVec	TNFerade: replication deficient adenovirus with TNF-a controlled by radiation induced promoter	Esophageal cancer	Phase 2
Shenzhen SiBiono GeneTech	rAd-p53: replication deficient adenovirus encoding hu recombinant p53	Advanced thyroid tumors, oral, maxillofacial tumors	Phase 4
AAV			
Applied Genetic Technologies (Alachua, FL, USA)	rAAV1-CB-hAAT: AAV with α-1-antitrypsin gene	α-1-antitrypsin deficiency	Phase 2
	rAAV2-CB-human retinal pigment epithelium-specific 65-dalton protein (RPE65)	Congenital amaurosis (blindness with mutation in RPE gene)	Phase 1/2
Amsterdam Molecular	AMT-101: AAV with human lipoprotein lipase (LPL) gene	LPL deficiencey	Phase 3?
Ceregene (San Diego)	CERE-120: AAV with neurotrophic factor, neuturin	Parkinson's disease	Phase 1/2
	CERE-110: AAV with gene for nerve growth factor	Alzheimer's disease	Phase 1/2
Celladon (La Jolla, CA, USA)	SERCA-2a: sarcoplasmic reticulum Ca_2 plus ATPase gene with AAV vector	Congestive heart failure	Phase 1/2
Genzyme	AAV2-sFLT01: AAV with anti-VEGF	Wet macular degeneration	Phase 1
Neurologix	NLX-P101: GAD in virus injected into subthalmic nucleus of the brain	Parkinson's disease	Phase 2 completed
Targeted Genetics (Seattle)	tgAAG76: AAV with hRPE65	Congenital amaurosis (blindness with mutation in RPE gene)	Phase 1/2
	tgAAC94: AAV2 with TNF-α-IgG1 fusion gene	Arthritis	Phase 2 completed
Plasmid			
AnGes (Tokyo)	Hepatocyte growth factor plasmid	Arterial disease	Phase 2
Genexine (Seoul, Korea)	GX-12: plasmid plus IL-12 mutant, given with HAART	HIV-AIDS	Phase 1
ScanCell (Nottingham, UK)	SCIB1: plasmid with gene for tyrosine related protein	Melanoma	Phase 1/2
Vical (San Diego)	Allovectin-7: plasmid with HLA-B7 and β-2 microglobulin genes, injected into tumors	Melanoma	Phase 3
ViroMed (Minnetonka, MN, USA)	VM202: plasmid with two isoforms of hepatocyte growth factor, HGF728 and HGF 723	Limb ischemia Myocardial ischemia	Phase 2 Phase 1/2
Other			
Diamyd Medical (Stockholm)	Nerve-targeting drug delivery system: HSV vector with enkaphalin administered intradermally	Pain	Phase 1
Epeius Biotechnologies (San Marino, CA, USA)	Rexin-G: nanoparticle delivering cyclin-G1 gene	Advanced pancreatic metastatic breast cancer, osteosarcoma, and soft tissue sarcoma	Phase 1/2
MultiGene Vascular Systems (Nesher, Israel)	Patient cells modified with four angiogenic genes	Peripheral artery disease	Phase 1/2

AAV, adeno-associated virus; HSV, herpes simplex virus

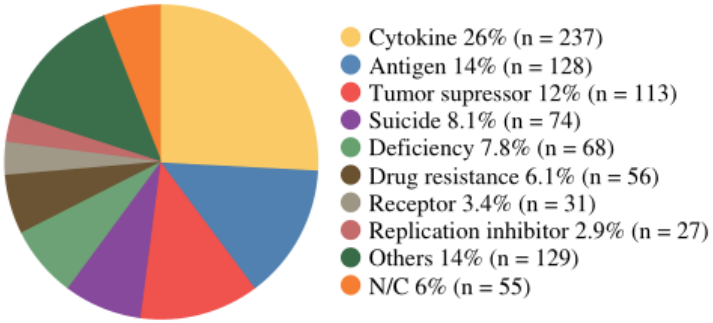


Figure 5. Distribution of gene therapy clinical trials by gene. N/C = not communicated

Nature Biotechnology, February 2011

Table 1 Some recent advances in clinical gene therapy

	Vector, dose range, and number and ages of patients	Transgene and promoter	Route of administration and cell target	Scientific and clinical outcomes	Reference
Gene therapy for genetic disease					
Leber's congenital amaurosis	AAV2; 1.5×10^{10} vg per patient; three patients (19–26 years old)	RPE65 under chicken β-actin promoter	Subretinal injection to retinal epithelial cells	All patients showed improved visual acuity and modest improvements in pupillary light reflexes.	3
	AAV2; 10^{11} vg per patient; three patients (17–23 years old)	RPE65 under cognate promoter	Subretinal injection to retinal epithelial cells	No change in visual acuity or retinal responses to flash or pattern electroretinography; microperimetry and dark-adapted perimetry showed no change in retinal function in patients 1 and 2 but showed improved retinal function in patient 3.	2
	AAV2; 1.5×10^{10}, 4.8×10^{10} or 1.5×10^{11} vg per patient; 12 patients (8–44 years old)	RPE65 under chicken β-actin promoter	Subretinal injection to retinal epithelial cells	All patients showed sustained improvement in subjective and objective measurements of vision (dark adaptometry, pupillometry, electroretinography, nystagmus and ambulatory behavior).	4
Hemophilia B	AAV8; 2×10^{11}, 6×10^{11} or 2×10^{12} vg per kg body weight; six patients (27–64 years old)	FIX gene, regulated by the human apolipoprotein hepatic control region and human α-1-antitrypsin promoter	Intravenous delivery targeting hepatocytes	Durable circulating FIX at 2–11% normal levels; decreased frequency (two of six patients) or cessation (four of six) of spontaneous hemorrhage	11
X-linked severe combined immunodeficiency (SCID-X1)	Gammaretrovirus; ten patients (4–36 months old); $CD34^+$ cells were infused (without conditioning) at doses of 60×10^6 to 207×10^6 cells per patient	Interleukin-2 receptor common γ-chain, retroviral LTR	*Ex vivo*, $CD34^+$ hematopoietic stem and progenitor cells	Functional polyclonal T-cell response restored in all patients; one patient developed acute T-cell lymphoblastic leukemia	23
	Gammaretrovirus; nine patients (1–11 months old); $CD34^+$ cells were infused (without conditioning) at doses of 1×10^6 to 22×10^6 cells per kg	Interleukin-2 receptor common γ-chain, retroviral LTR	*Ex vivo*, $CD34^+$ hematopoietic stem and progenitor cells	Functional T-cell numbers reached normal ranges. Transduced T cells were detected for up to 10.7 years after gene therapy. Four patients developed acute T cell lymphoblastic leukemia, one died.	24
Adenosine deaminase deficiency resulting in severe combined immunodeficiency (ADA-SCID)	Gammaretrovirus; six patients (6–39 months old); $CD34^+$ cells were infused (after non-myeloablative conditioning with melphalan (Alkeran), 140 mg per m^2 body surface area, or busulfan (Myleran), 4 mg per kg) at doses of $<0.5 \times 10^6$ to 5.8×10^6 cells per kg	Adenosine deaminase gene, retroviral LTR	*Ex vivo*, $CD34^+$ hematopoietic stem and progenitor cells	Restoration of immune function in four of six patients; three of six taken off enzyme-replacement therapy; four of six remain free of infection	25
	Gammaretrovirus; ten patients (1–5 months old); $CD34^+$ cells were infused (after non-myeloablative conditioning with busulfan, 4 mg per kg) at doses of 3.1×10^6 to 13.6×10^6 cells per kg	Adenosine deaminase gene, retroviral LTR	*Ex vivo*, $CD34^+$ hematopoietic stem and progenitor cells	Nine of ten patients had immune reconstitution with increases in T-cell counts (median count at 3 years, $1.07 \times 10^9\ l^{-1}$) and normalization of T-cell function. Eight of ten patients do not require enzyme-replacement therapy.	26
Chronic granulomatous disorder	A range of studies, using gammaretrovirus vectors pseudotyped either with gibbon ape leukemia virus envelope or with an amphotrophic envelope; various non-myeloablative conditioning strategies	Gp91phox, retroviral LTR	*Ex vivo*, $CD34^+$ hematopoietic stem and progenitor cells	Twelve of twelve patients showed short-term functional correction of neutrophils with resolution of life-threatening infections. Three patients developed myeloproliferative disease.	27*
Wiskott-Aldrich syndrome	Gammaretrovirus; ten patients; $CD34^+$ cells were infused (after non-myeloablative conditioning with busulfan, 4 mg per kg)	WAS gene, retroviral LTR	*Ex vivo*, $CD34^+$ hematopoietic stem and progenitor cells	Nine of ten patients showed improvement of immunological function and platelet count. Two patients developed acute T-cell lymphoblastic leukemia.	28, 29
β-thalassemia	Self-inactivating HIV-1–derived lentivirus; one patient (18 years old) received fully myeloablative conditioning with busulfan; 3.9×10^6 $CD34^+$ cells per kg	Mutated adult β-globin ($\beta^{A(T87Q)}$) with anti-sickling properties, LCR control	*Ex vivo*, $CD34^+$ hematopoietic stem and progenitor cells	Patient has been transfusion independent for 21 months. Blood hemoglobin is maintained between 9 and 10 g dl^{-1}, of which one-third contains vector-encoded β-globin.	30
Adrenoleukodystrophy	Self-inactivating HIV-1–derived lentivirus; two patients (7 and 7.5 years old) received myeloablative conditioning with cyclophosphamide (Cytoxan) and busulfan; transduced $CD34^+$ cells, 4.6×10^6 and 7.2×10^6 cells per kilogram, respectively	Wild-type *ABCD1* cDNA under the control of the MND viral promoter	*Ex vivo*, $CD34^+$ hematopoietic stem and progenitor cells	9–14% of granulocytes, monocytes, and T and B lymphocytes expressing the ALD protein; beginning 14–16 months after infusion of the genetically corrected cells, progressive cerebral demyelination in the two patients attenuated.	8

Recent Advances in Gene Therapy Clinical Trials Part 1

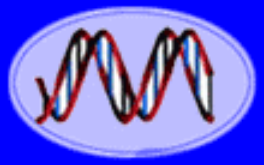
Nature Biotechnology, July 2012

Table 1 Some recent advances in clinical gene therapy (continued)

	Vector, dose range, and number and ages of patients	Transgene and promoter	Route of administration and cell target	Scientific and clinical outcomes	Reference
Gene therapy for genetic disease					
Duchenne muscular dystrophy	Phosphorodiamidate morpholino antisense oligodeoxynucleotides; dose escalation from 0.5 to 20.0 mg per kg; 19 patients (5–15 years old)	Oligonucleotide promotes skipping of spliceosome across diseased exon 51 of dystrophin gene	i.v., aiming to promote exon skipping in muscle cells	No serious treatment-related toxicities; muscle biopsies showed exon 51 skipping in all cohorts and dose-dependent expression of new dystrophin protein at doses of 2 mg per kg and above. Best responder had 18% normal muscle dystrophin levels.	9
Gene therapy for degenerative disease					
Heart failure	AAV1; 6×10^{11}, 3×10^{12} or 1×10^{13} DNase-resistant particles per patient	Sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2a), CMV immediate early promoter	Antegrade epicardial coronary artery infusion over a 10-min period, targeting cardiac myocytes	High dose showed significant improvement in symptoms, functional status, biomarker (N-terminal prohormone brain natriuretic peptide) and left ventricular function, plus significant improvement in clinical outcomes.	11
Gene therapy for cancer					
B-cell leukemia and lymphoma	Self-inactivating lentivirus expressing a chimeric T cell receptor; a single patient was conditioned with pentostatin (Nipent; 4 mg per m^2) and cyclophosphamide (600 mg per m^2) before receiving 1.5×10^5 transduced T cells per kg (total 3×10^8 T cells, of which 5% were transduced)	Anti-CD19 scFv derived from FMC63 murine monoclonal antibody, human CD8α hinge and trans-membrane domain, and human 4-1BB and CD3ζ signaling domains	*Ex vivo*, autologous T cells, i.v. infusion, split over 3 d	Transduced T cells expanded more than 1,000 times *in vivo*, with delayed development of the tumor lysis syndrome and complete remission, ongoing 10 months after treatment. Engineered cells persisted at high levels for 6 months in the blood and bone marrow.	31
	Murine stem cell virus–based splice-gag (retroviral) vector expressing CD19 CAR; eight patients (47–63 years old) with progressive B-cell malignancies received cyclophosphamide and fludarabine (Fludara) before CAR-transduced autologous T cells and interleukin 2. Patients received 0.3×10^7 to 3.0×10^7 CAR^+ T cells per kg, of which an average of 55% were transduced.	Anti-CD19 scFv derived from the FMC63 mouse hybridoma, a portion of the human CD28 molecule and the intracellular component of the human TCR-ζ molecule	*Ex vivo*, autologous T cells, single i.v. infusion, followed (3 h) by a course of IL2	Varied levels of anti–CD19-CAR–transduced T cells could be detected in the blood of all patients. One patient died on trial, with influenza A pneumonia, nonbacterial thrombotic endocarditis and cerebral infarction. Four patients had prominent elevations in serum levels of IFNγ and TNF, correlating with severity of acute toxicities. Six of the eight patients treated obtained objective remissions.	32
Acute leukemia	SFG retrovirus expressing an inducible suicide system for improved safety of stem cell transplantation to prevent graft-versus-host disease (GVHD); transduced haploidentical T cells (1×10^6 to 1×10^7 T cells per kg); five patients (3–17 years old)	FK506-binding protein linked to modified human caspase 9 with truncated CD19 as a selectable marker; in the presence of the drug, the iCasp9 promolecule dimerizes and activates apoptosis; retroviral LTR	*Ex vivo*, allodepleted haploidentical T cells, infused i.v. into recipients of allogeneic bone marrow transplants.	The genetically modified T cells were detected in peripheral blood from all five patients and increased in number over time. A single dose of dimerizing drug, given to four patients in whom GVHD developed, eliminated more than 90% of the modified T cells within 30 min after administration and ended the GVHD without recurrence.	33
Squamous-cell carcinoma of the head and neck	Oncolytic vaccine based on herpes virus combined with chemotherapy and chemoradiotherapy; patients with stage III, stage IVA or stage IVB disease; four doses of virus, 10^6–10^8 p.f.u. per dose	Clinical isolate of HSV-1 from which the proteins ICP34.5 and ICP47 have been deleted	Intratumoral injection into nodules of squamous head and neck carcinoma	14 patients (82.3%) showed tumor response by RECIST criteria, and pathologic complete remission was confirmed in 93% of patients at neck dissection. Prolonged progression-free survival was seen in two-thirds of the patients.	34
Melanoma	Oncolytic vaccine based on herpes virus; patients with stage IIIc and IV disease; 4×10^6 p.f.u. followed 3 weeks later by up to 4×10^8 p.f.u. every 2 weeks for up to 24 treatments	Clinical isolate of HSV-1 from which the proteins ICP34.5 and ICP47 have been deleted	Intratumoral injection into melanoma nodules	The overall response rate by RECIST was 26%, with regression of both injected and distant (including visceral) lesions. 92% of the responses had been maintained for 7 to 31 months. Ten additional patients had stable disease for >3 months, and two additional patients had surgical complete response.	35
Advanced or metastatic solid tumors refractory to standard of care treatment, or for which no curative standard therapy existed	25 adult patients received 75 mg per m^2 docetaxel (Taxotere; day 1) and escalating doses of reovirus up to 3×10^{10} $TCID_{50}$ (days 1–5) every 3 weeks	Reovirus type 3 Dearing, a wild-type double-stranded RNA virus	Intravenous delivery to treat advanced and/or disseminated cancer	Of 16 evaluable patients, dose-limiting toxicity of grade 4 neutropenia was seen in one patient but the maximum tolerated dose was not reached. Antitumor activity was seen with one complete response and three partial responses. A disease-control rate (combined complete response, partial response and stable disease) of 88% was observed.	18

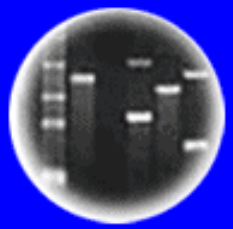
Recent Advances in Gene Therapy Clinical Trials Part 2

Nature Biotechnology, July 2012



DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



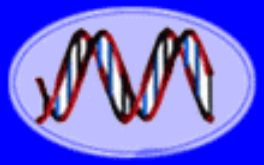
DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

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



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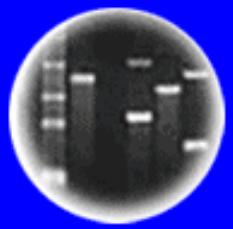


DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

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?"

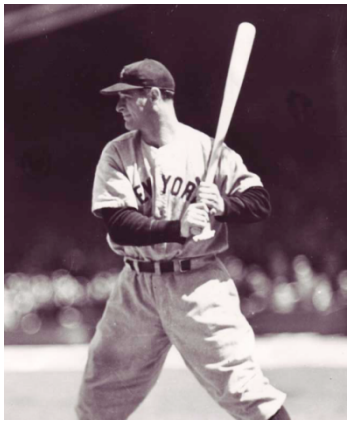
a. Yes
b. No

Some Issues With Human Gene Therapy

- Regulation
- Consent
- Risks
- Enhancement
- Eugenics (Germ Line)
- Availability To Everyone

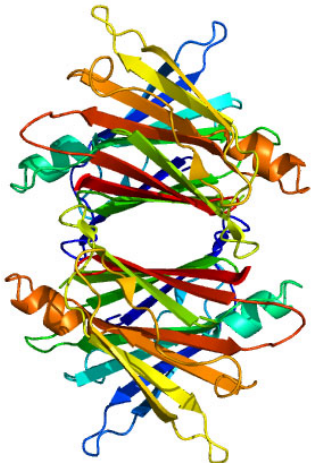
The Frontiers of Human Gene Therapy: RNAi "Drugs", Vaccines, Therapeutic Cloning + Gene Therapy

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



Lou Gehrig's Disease - Amyotrophic Lateral Sclerosis (ALS)

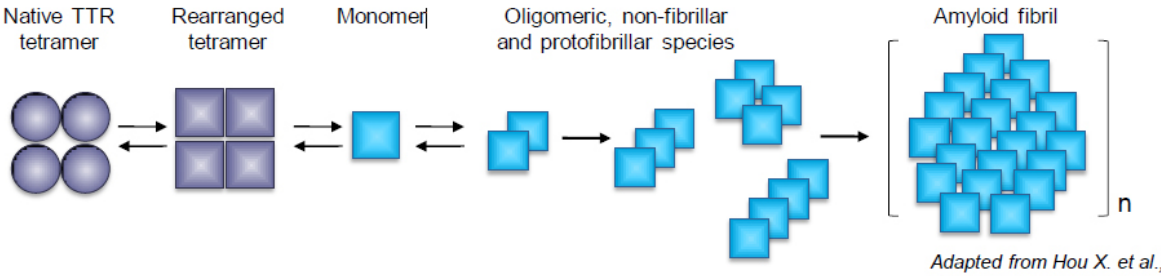
- One cause is a dominant mutation in the coding region of the *superoxide dismutase (SOD1)* gene (SOD is an anti-oxidant)
- Mutant SOD1 Protein is Toxic to Motor Neurons



transthyretin

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

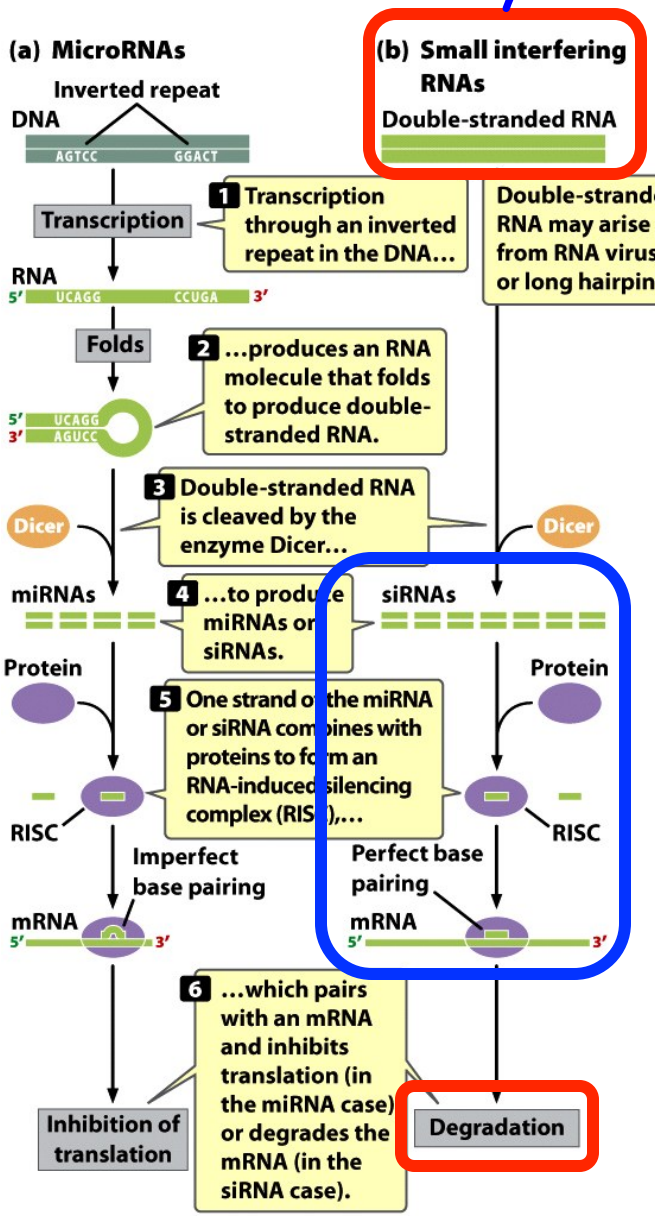


Adapted from Hou X. et al., 2007

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



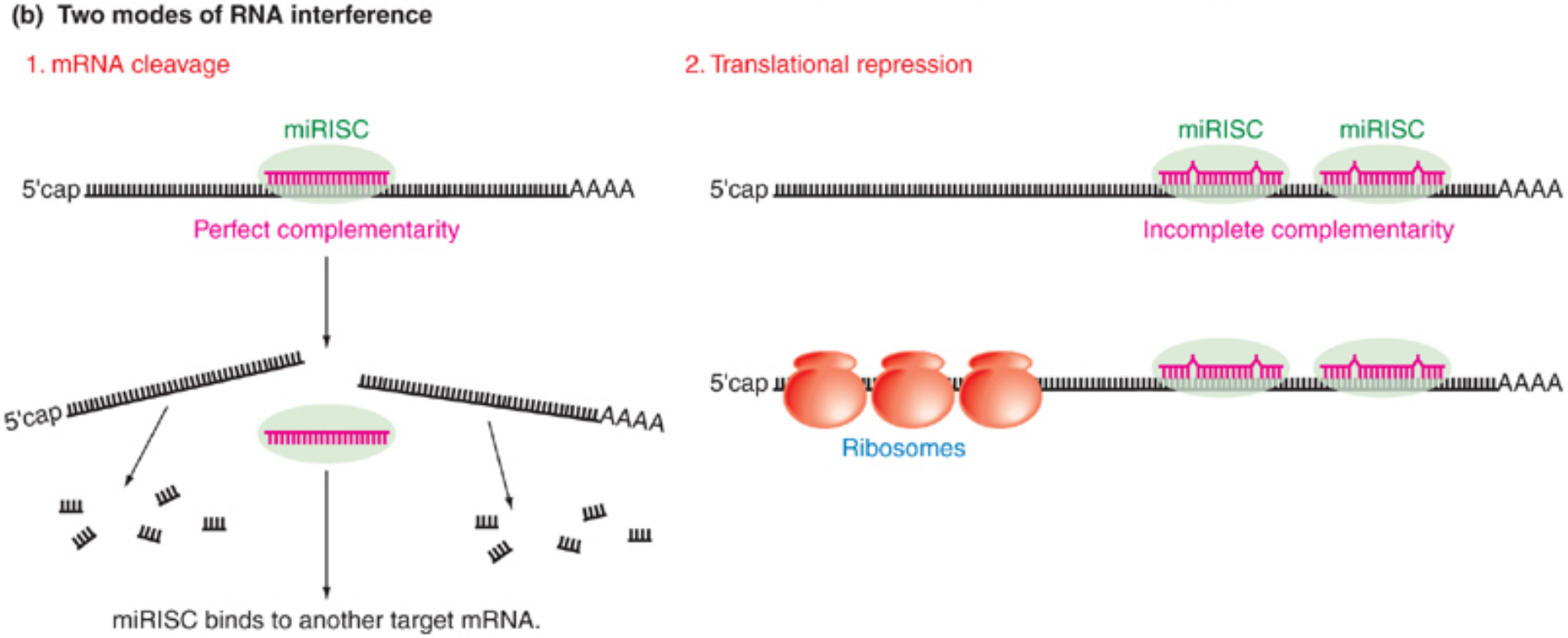
siRNA

Target mRNA

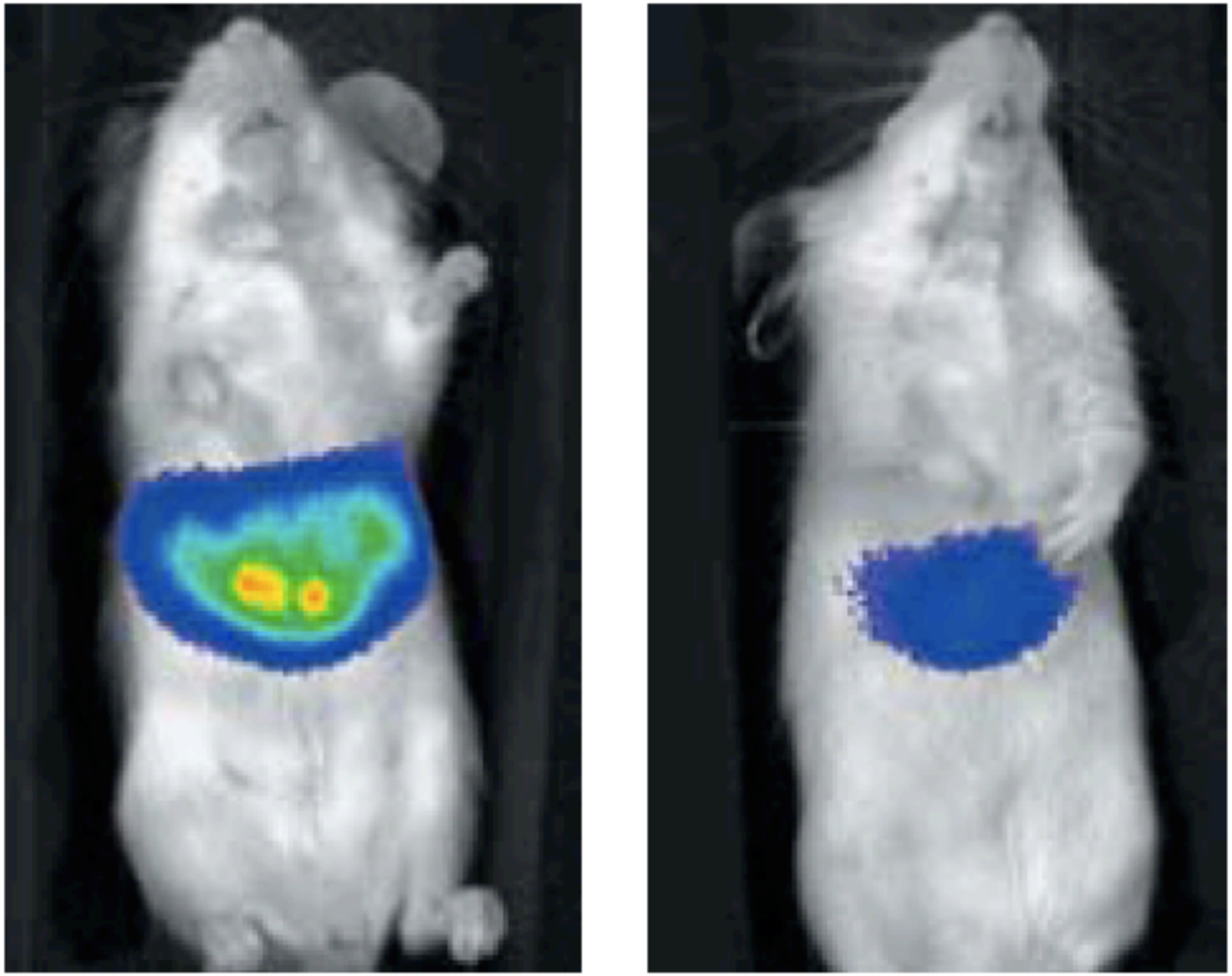
Figure 14-22
Genetics: A Conceptual Approach, Third Edition

Andrew Fire & Craig Mello Nobel Prize-2006

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



Using RNAi To Inhibit Gene Activity



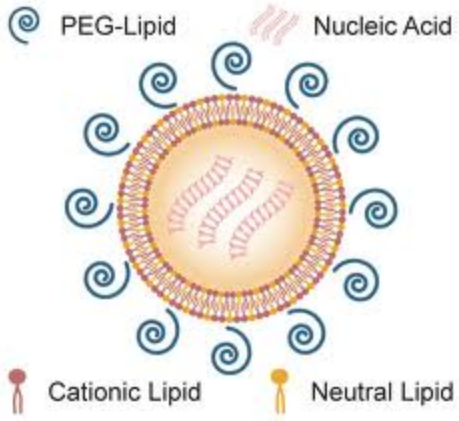
MICE LIGHT UP when injected with DNA containing the luciferase gene (*left*). But scientists took the shine off the mice by also injecting siRNAs that match the gene (*right*), thus demonstrating one way to exploit RNAi in mammals.

RNAi Gene Therapy for Transthyretin-mediated Amyloidosis

- **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

Alnylam PHARMACEUTICALS

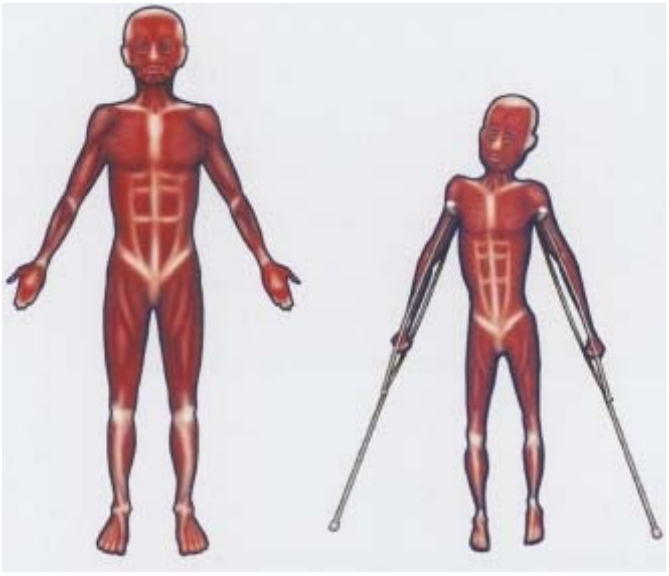
Lipid Nanocarrier



Molecular Drug for Muscular Dystrophy

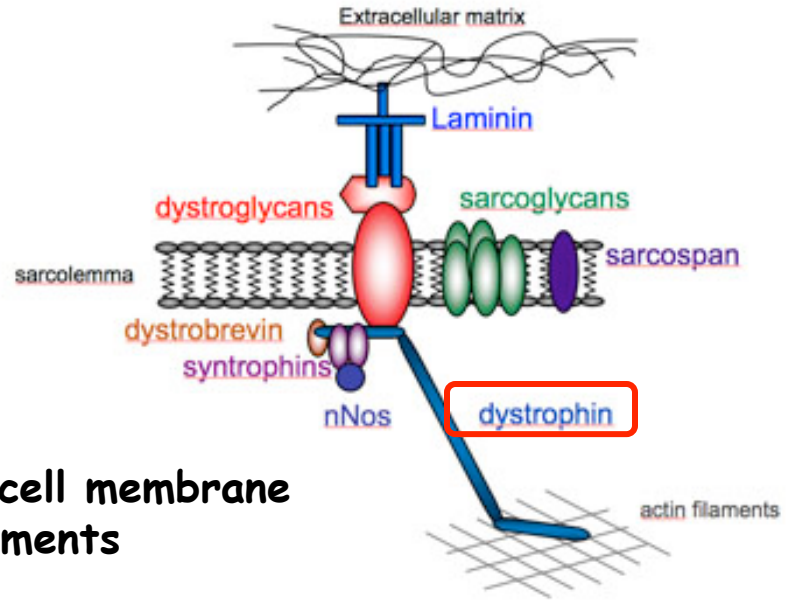
Duchenne Muscular Dystrophy

- 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



Normal healthy musculature

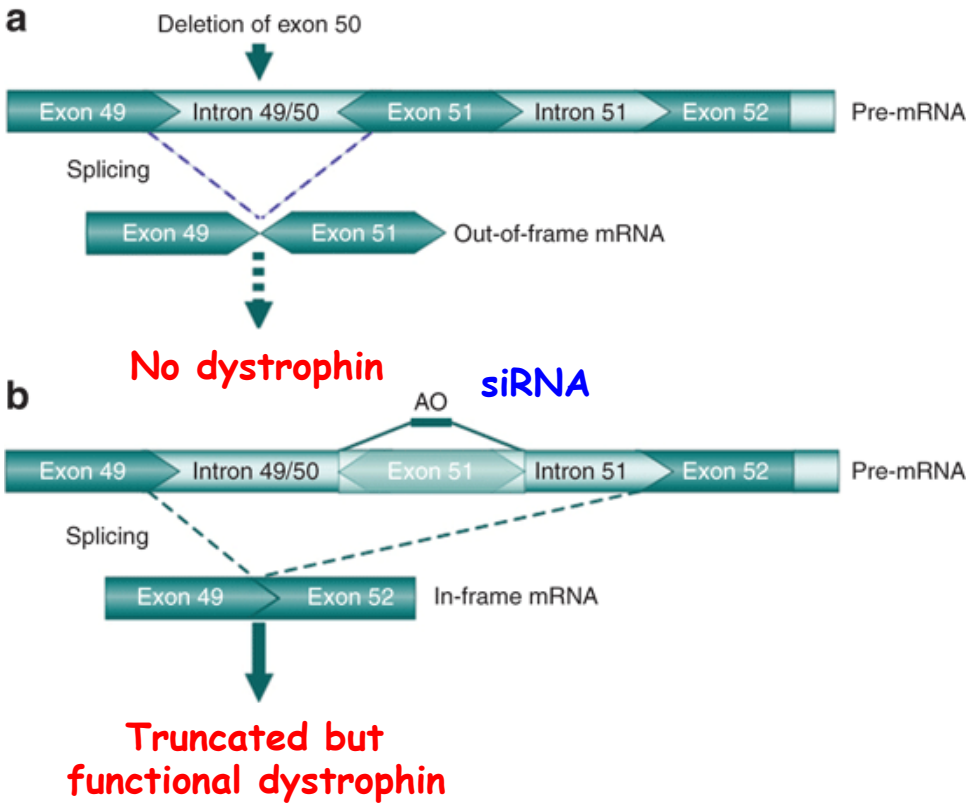
Muscular dystrophy



Dystrophyin links the cell membrane with actin filaments

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%



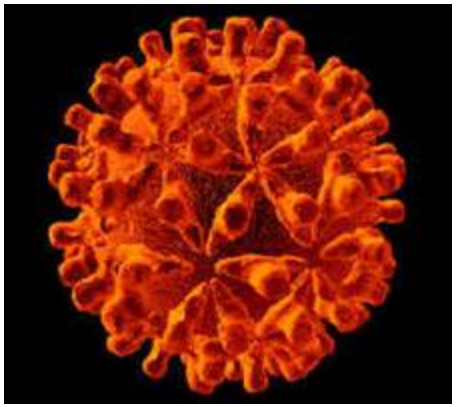
Vaccine for the Hepatitis C Virus

Hepatitis C

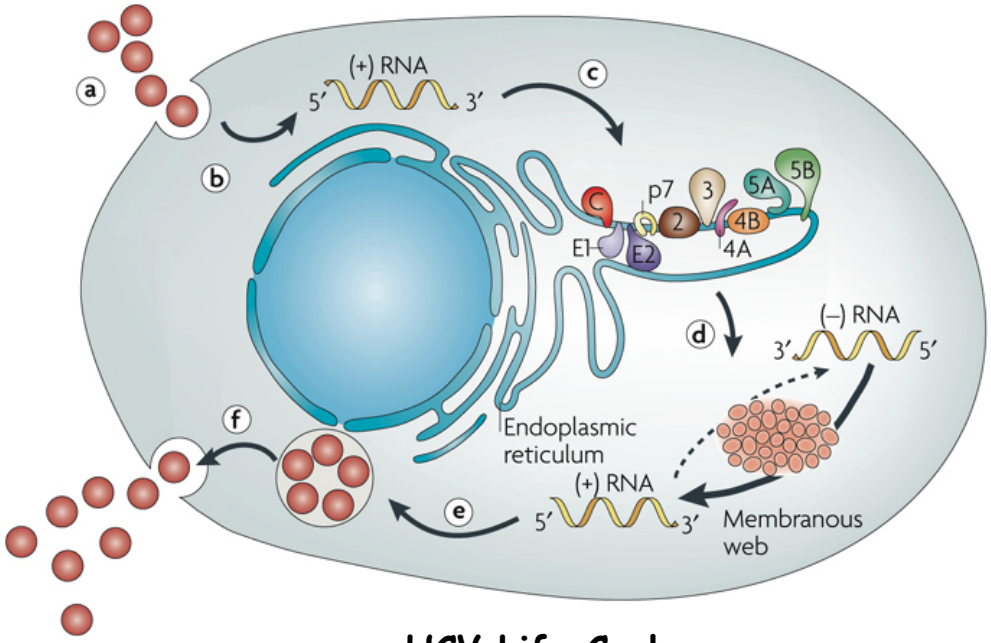
- Caused by the Hepatitis C virus (HCV), a single-stranded RNA virus
- HCV infects the liver. Chronic infection can lead to cirrhosis, liver failure, or liver cancer
- Replication of HCV requires microRNA 122 (similar to siRNA)

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



Hepatitis C Virus



HCV Life Cycle

Promising Future of Human Gene Therapy

nature medicine

Rescue of hearing and vestibular function by antisense oligonucleotides in a mouse model of human deafness

IN brief

First gene therapy approved

Jörn Aldag, uniQure CEO

The first person to be administered a commercial gene therapy will be treated in Germany in the middle of 2013. The European Commission granted marketing authorization for Glybera (alipogene tiparvovec) for the treatment

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 Periasamy[1] and Anuradha Kalyanasundaram[1]

DECEMBER 2012 NATURE BIOTECHNOLOGY

Companies in footrace to deliver RNAi

PLOS | BLOGS

Monday, February 25, 2013 | Genetics in context

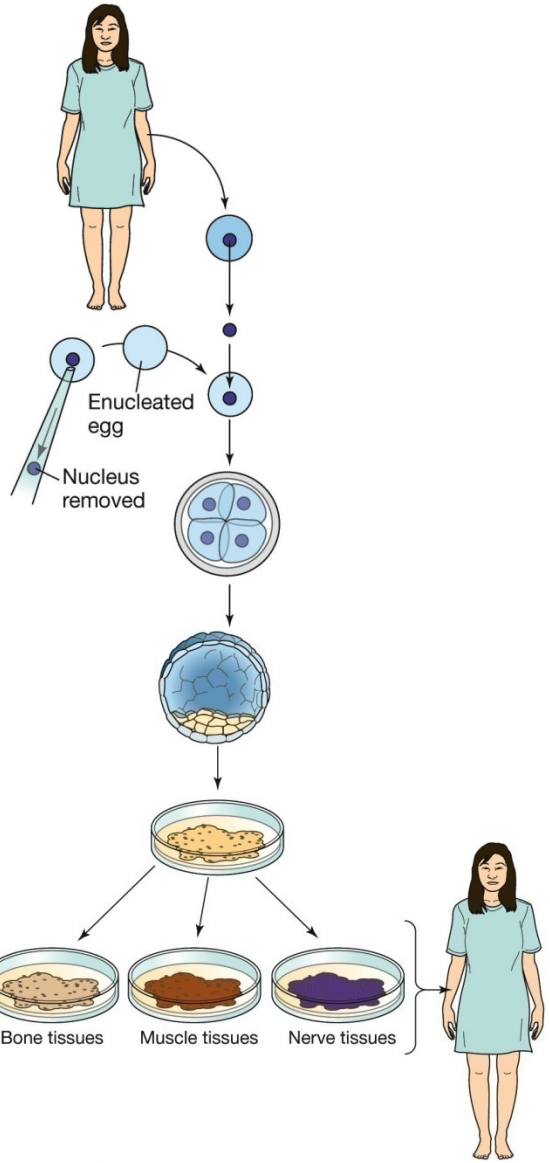
Gene Therapy for Canavan Disease: Max's Story

By Ricki Lewis, PhD
Posted: December 19, 2012

Neuron

Restoration of Hearing in the VGLUT3 Knockout Mouse Using Virally Mediated Gene Therapy

Combining Gene Therapy With Stem Cells & Therapeutic Cloning in the Future

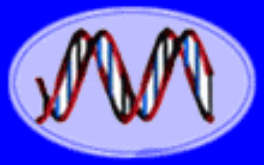


LIFE 8e, Figure 19.8 (I

LIFE 8e, Figure 19.8 (Part 2)

Genetic Engineer Cells Before Nuclear or Cell Transfer

Example Defective Insulin Gene in Pancreas



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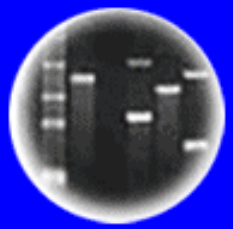

DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

The End!!

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