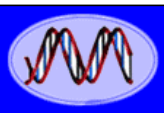
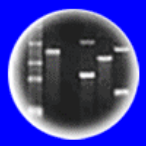

DNA
Genetic Code of Life


Entire Genetic Code of a Bacteria


DNA Fingerprinting


Cloning: Ethical Issues and Future Consequences


Plants of Tomorrow

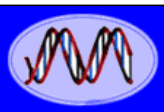
HC70A & SAS70A Spring 2017
Genetic Engineering in Medicine, Agriculture, and Law

Professors Bob Goldberg & John Harada

Lecture 8
Human Genetic Engineering and Gene Therapy

UCLA

UCDAVIS
UNIVERSITY OF CALIFORNIA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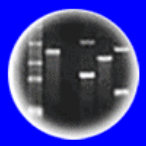

DNA Fingerprinting


Cloning: Ethical Issues and Future Consequences


Plants of Tomorrow

THEMES

Human Genetic Engineering and Gene Therapy

1. What is Gene Therapy?
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)
   a. Vectors
   b. Some Problems and Improvements with Gene Therapy
4. *In Vivo* Gene Therapy
5. Gene Therapy to Control Cancer
6. Regulation of Gene Therapy
7. Issues Concerning Gene Therapy
8. Gene Editing & Human Gene Therapy

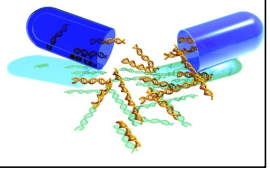
Genetically Engineered Organisms & Their Uses

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

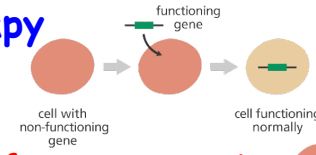
What is Gene Therapy?

- 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*

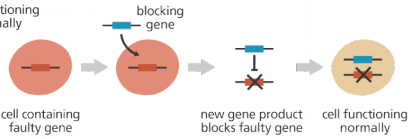


Types of Gene Therapy

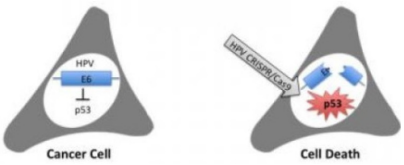
- Germline gene therapy
- Somatic gene therapy
  - Gene augmentation



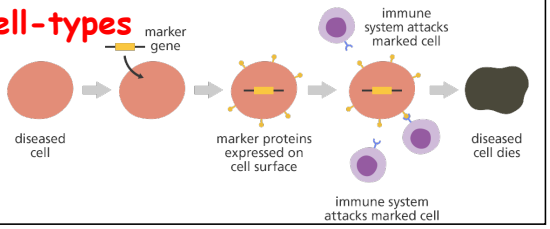
  - Targeted silencing of gene expression



  - Gene alteration
  - Gene replacement



  - Targeted killing of specific cell-types



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.

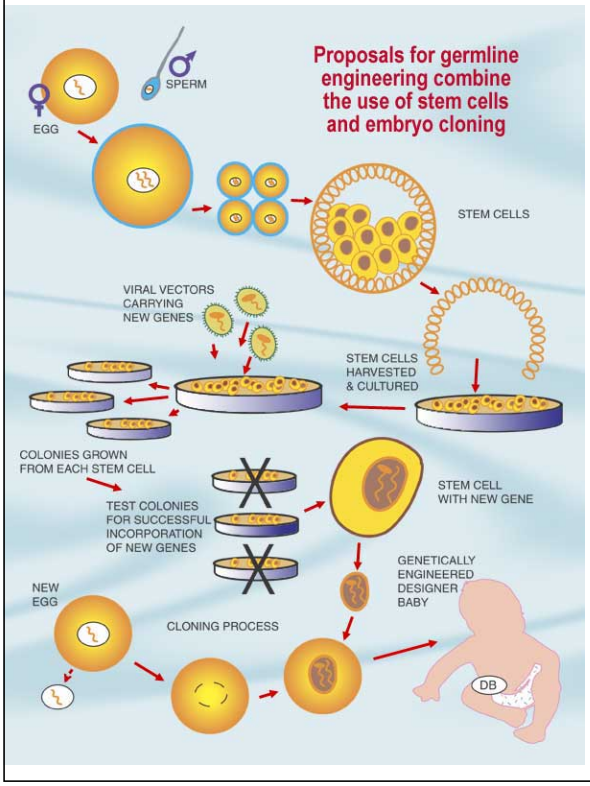
c. ▸ **Targeted inhibition of gene expression** is especially relevant in infectious disease, where essential functions of the pathogen are targeted. It could also be used to silence activated oncogenes in cancer, to damp down unwanted responses in autoimmune disease and maybe to silence a gain-of-function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

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

Issues Involved with Use of Gene Therapy

- Regulation
- NIH Guidelines
- Human Experimentation
- Ethics
- Eugenics
- Availability & Costs

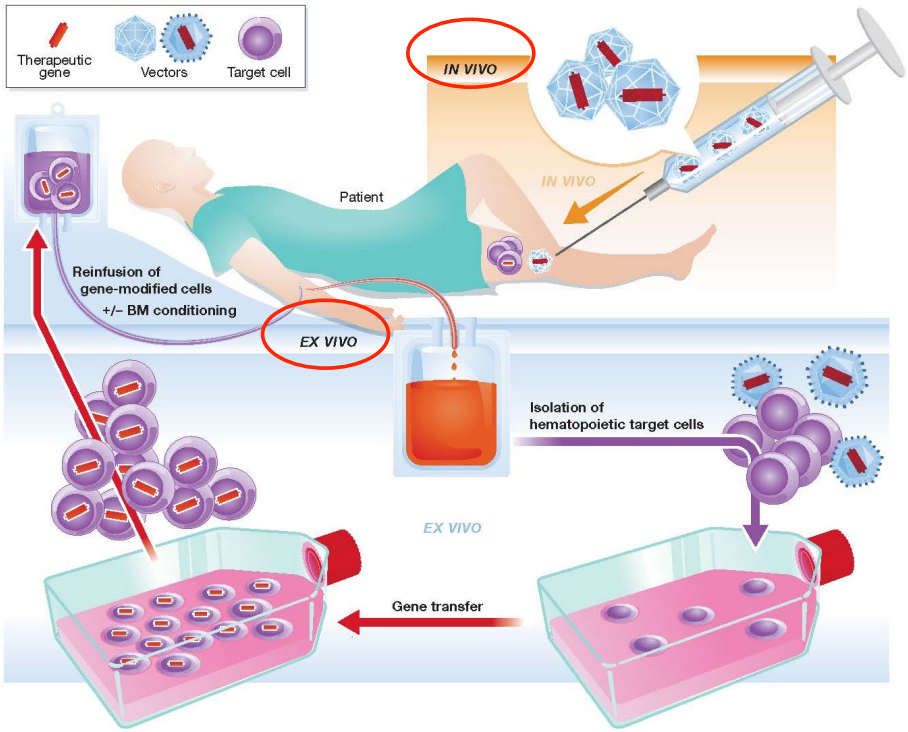
Germline Gene Therapy



- Germline gene therapy is when DNA is transferred into the cells that produce reproductive cells, eggs or sperm, in the body. This type of therapy allows for the correction of disease-causing gene variants that are certain to be passed down from generation to generation
- It is NOT ILLEGAL to conduct human germline gene therapy in the US – however, experiments using federal funding must be approved by the Recombinant DNA Advisory Committee and use by public and private labs requires FDA approval

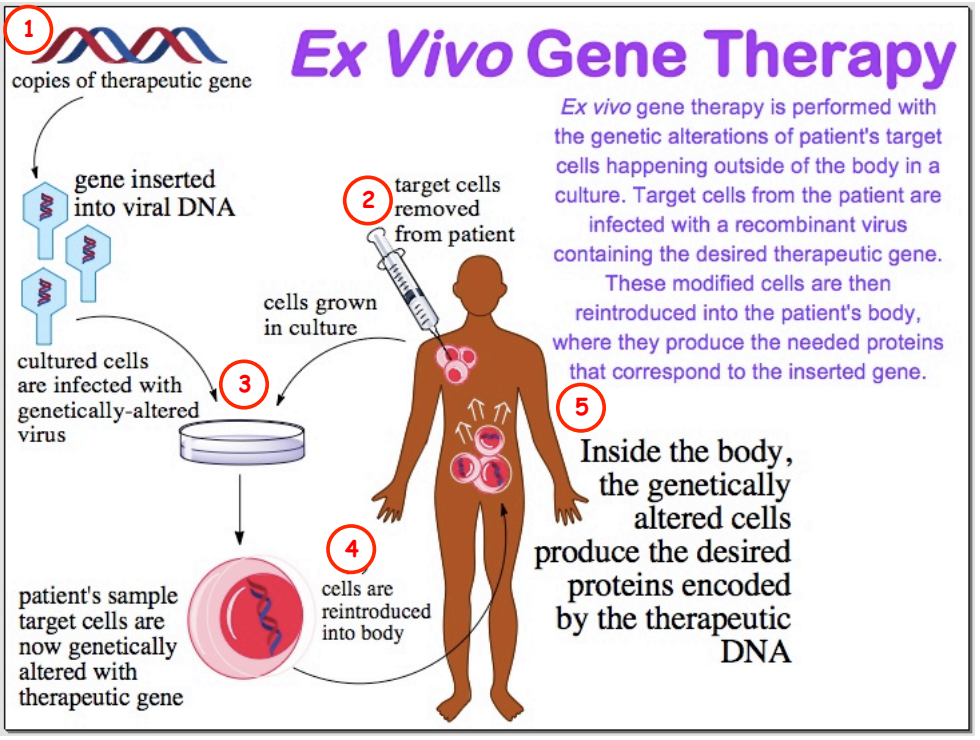
Somatic Cell Gene Therapy - *Ex Vivo* and *In Vivo*

Somatic cell - any cell of a living organism other than the reproductive cells



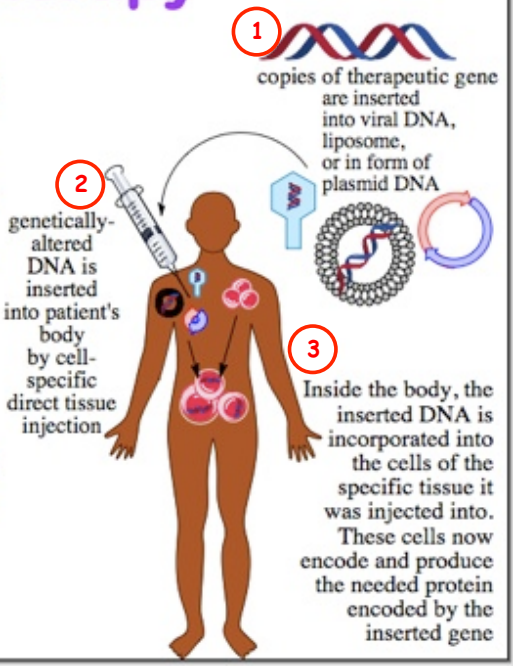
Ex Vivo Gene Therapy

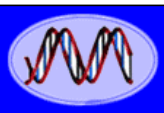
Ex vivo gene therapy is performed with the genetic alterations of patient's target cells happening outside of the body in a culture. Target cells from the patient are infected with a recombinant virus containing the desired therapeutic gene. These modified cells are then reintroduced into the patient's 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.





DNA
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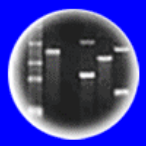
Entire Genetic Code of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

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

Questions to Consider Before Initiating Gene Therapy

1. What is known about the biology of the disorder?
2. Does the condition result from a mutation of one or more genes?
3. Has the affected 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?

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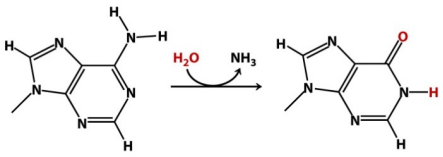
5. Can you deliver the gene to cells of the affected tissue?

Toolkit for Ex Vivo Somatic Cell Gene Therapy Procedures

1. Cloned copy of the therapeutic gene
2. Appropriate switch - often a strong switch to drive high level expression of the gene
3. Vector to transfer the gene into the cells
4. Autologous (obtained from the same individual) or non-autologous cells

Severe Combined Immunodeficiency Disease (SCID)
Adenosine Deaminase Gene (*ADA*) Deficiency

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

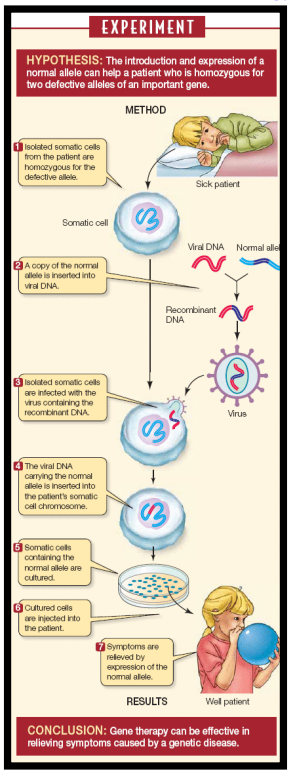


Degradation of Purine

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
- ADA-SCID 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

Ashanthi DeSilva

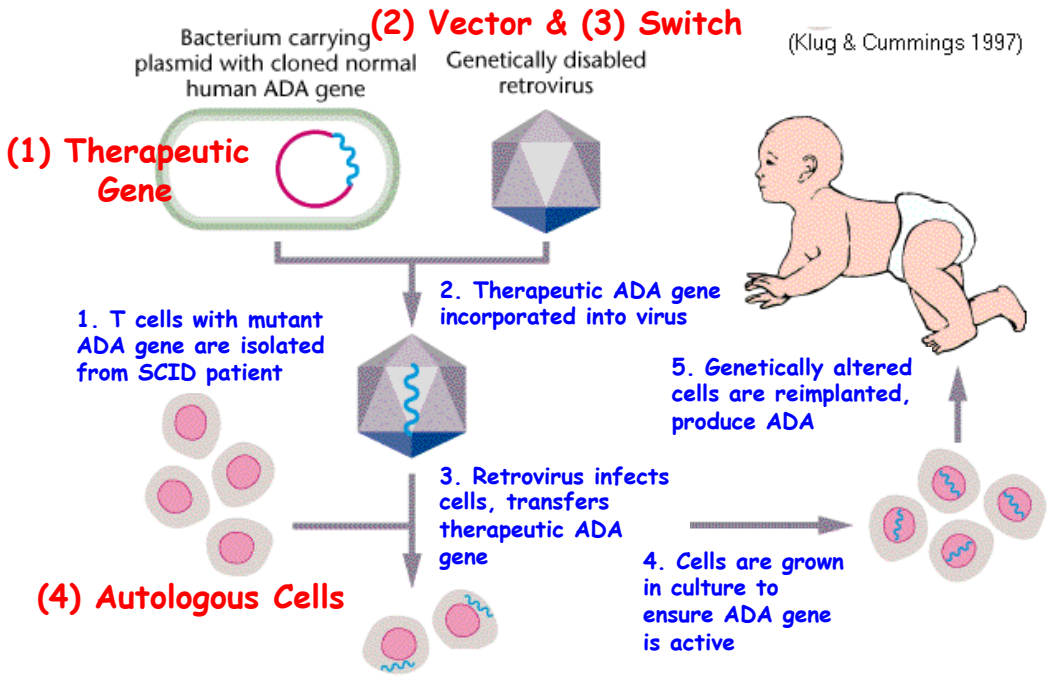
The new england journal of medicine

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

Gene Therapy for Immunodeficiency Due to Adenosine Deaminase Deficiency

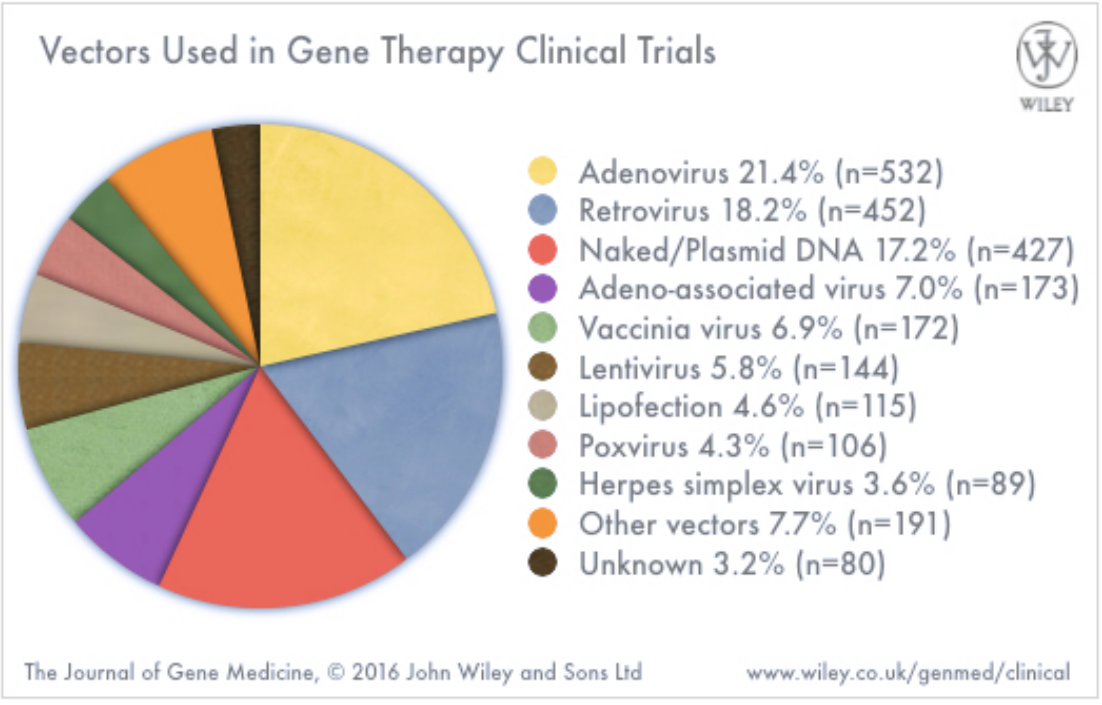
Gene Therapy with the Adenosine Deaminase (ADA) Gene

Ex Vivo Gene Therapy for Severe Combined Immunodeficiency (SCID)



ADA-SCID Clinical Trial Started in 1990

How are Therapeutic Genes Targeted and Delivered to Cells of Interest – with Vectors



Vectors Used to Deliver Genes to Cells in Gene Therapy

Table 19.3 Vectors used in gene therapy

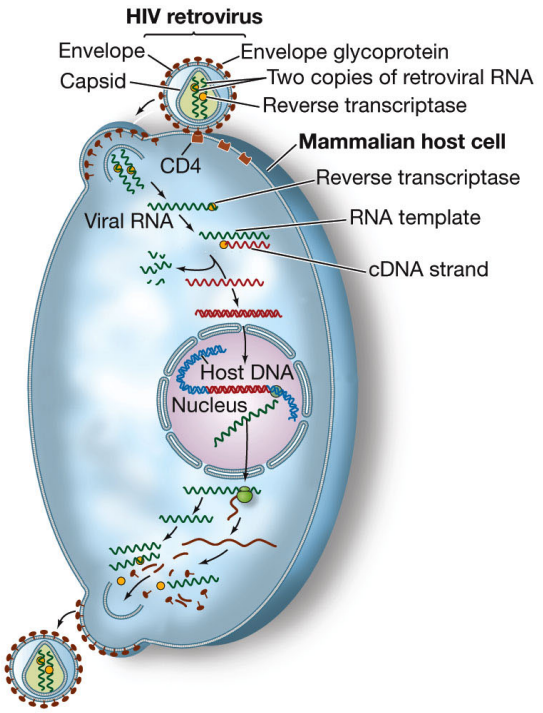
Vector	Advantages	Disadvantages
Retrovirus	Efficient transfer	Transfers DNA only to dividing cells, inserts randomly; risk of producing wild-type viruses
Adenovirus	Transfers to nondividing cells	Causes immune reaction
Adeno-associated virus	Does not cause immune reaction	Holds small amount of DNA; hard 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

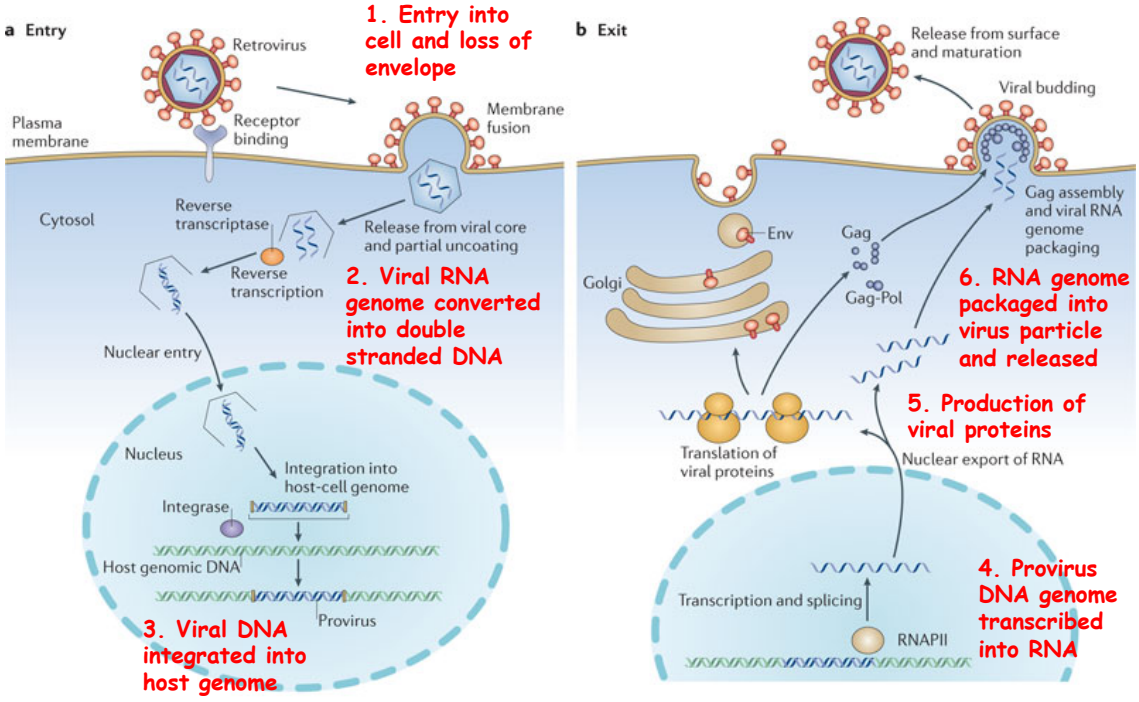
HIV is a Retrovirus

T-Cell



LIFE 8e, Figure 13.6

Retrovirus Life Cycle



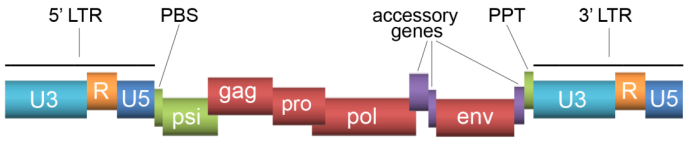
Nature Reviews | Microbiology

Retroviruses Replicate Using Reverse Transcriptase

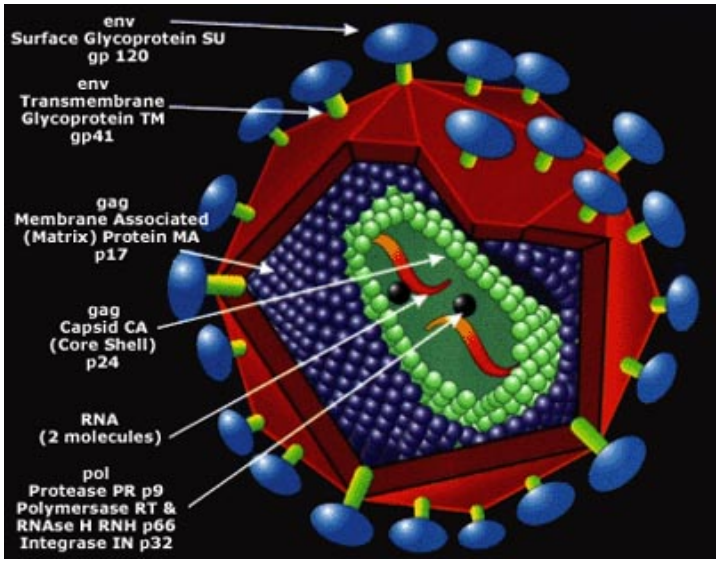
David Baltimore & Howard Temin-Nobel Prize 1975

Modification of the Central Dogma of Molecular Biology - RNA to DNA to RNA to Protein

Retrovirus Genome



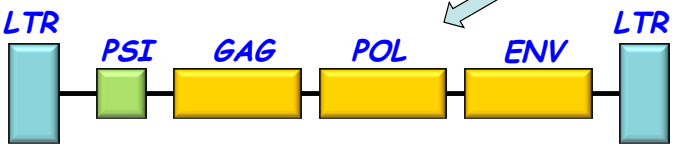
- 5' long terminal repeat (LTR) – switch
- 3' LTR – switch & transcriptional termination
- psi (Ψ) – packaging element needed to package the RNA genome into the viral particle
- gag – structural (coat) proteins
- pro - protease
- pol – reverse transcriptase
- env – envelope proteins

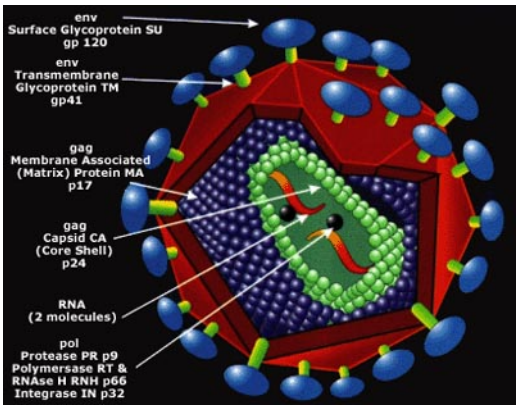


Retroviral Vector

THERAPEUTIC GENE

Retrovirus





Retroviral Vector

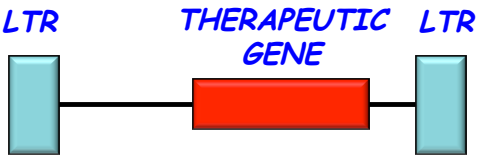
THERAPEUTIC GENE

Retrovirus

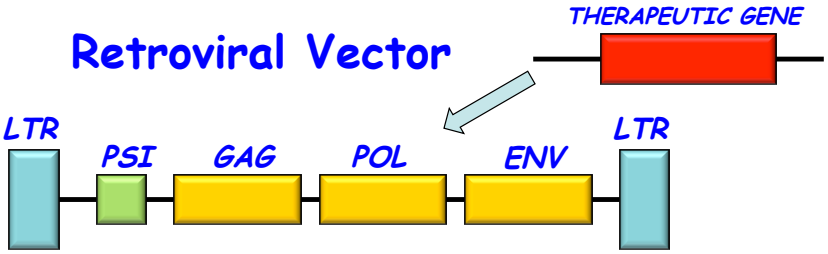
LTR
PSI
GAG
POL
ENV
LTR

env
Surface Glycoprotein SU
gp 120
env
Transmembrane
Glycoprotein TM
gp41
gag
Membrane Associated
(Matrix) Protein MA
p17
gag
Capsid CA
(Core Shell)
p24
RNA
(2 molecules)
pol
Protease PR p9
Polymersase RT &
RNAse H RNH p66
Integrase IN p32

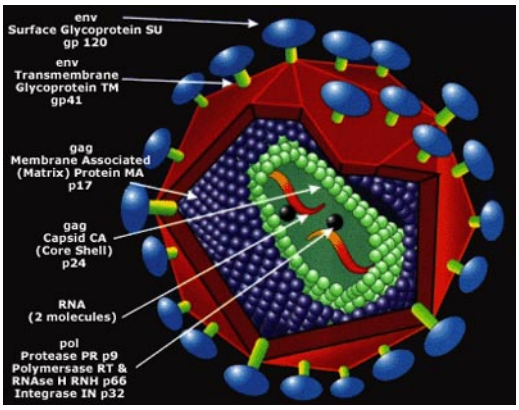
Retroviral Gene Therapy Vector



Retroviral Vector

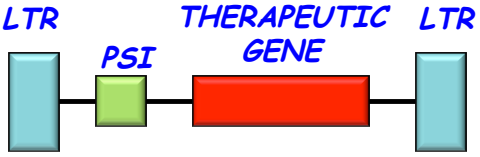


Retrovirus

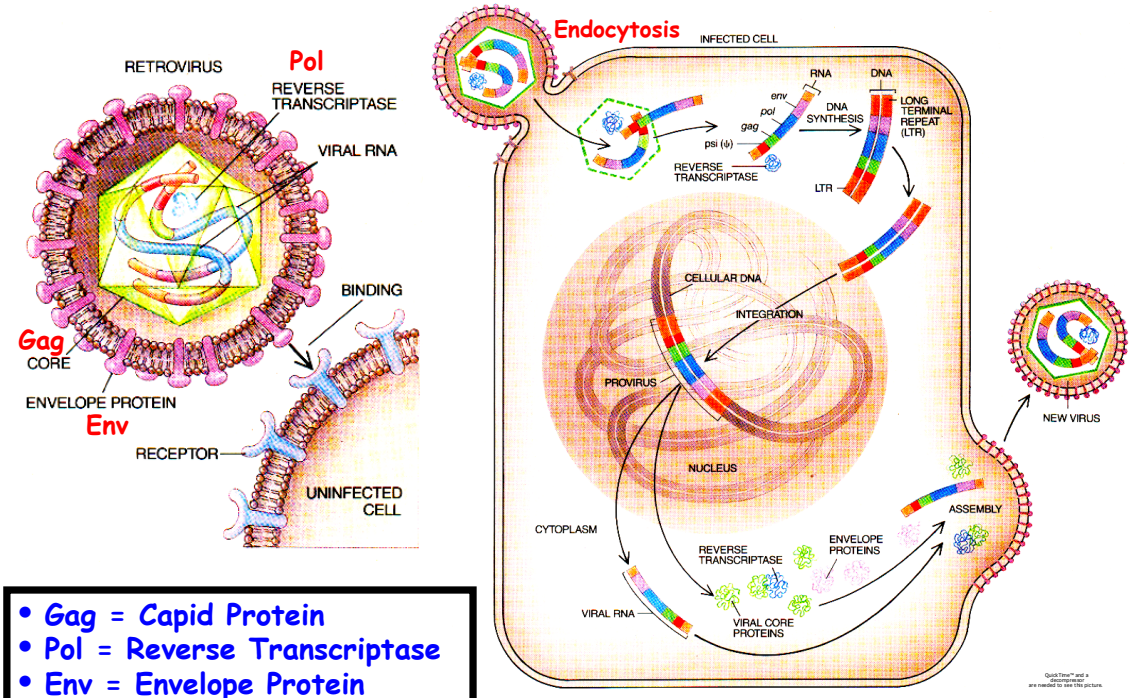


PSI is a packaging element – a RNA sequence that is required for the viral RNA to be packaged into the virus

Retroviral Gene Therapy Vector

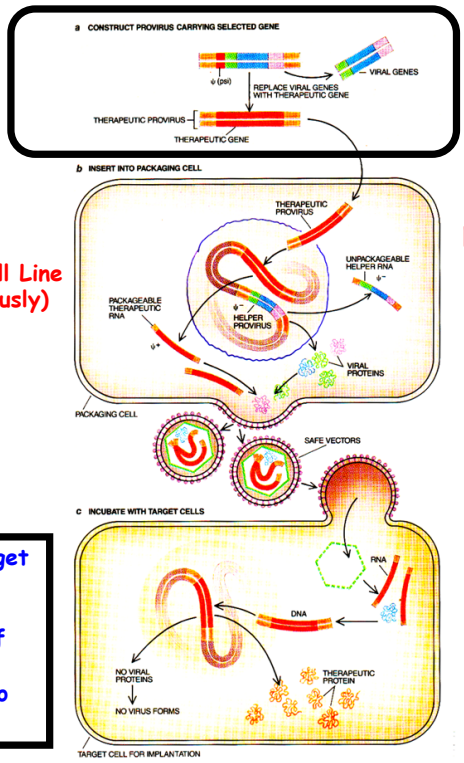


Using a Retrovirus as a Vector For Human Ex Vivo Gene Therapy



- 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

Packaging Cell Line (Made Previously)

B.

1. Packaging Cells Makes Viral Proteins
2. Cannot Package (Provirus Minus Ψ)
3. Packages Therapeutic Transcript (RNA contains Ψ)

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.

C.

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

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
- But - patients remained on ADA enzyme replacement therapy throughout the gene therapy treatment

Ashanthi DeSilva

Setbacks for Gene Therapy

The New York Times 1999

The Biotech Death of Jesse Gelsinger

By Sheryl Gay Stolberg
Published: November 28, 1999

- Gelsinger had a mild form of ornithine transcarbamylase (OTC) 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

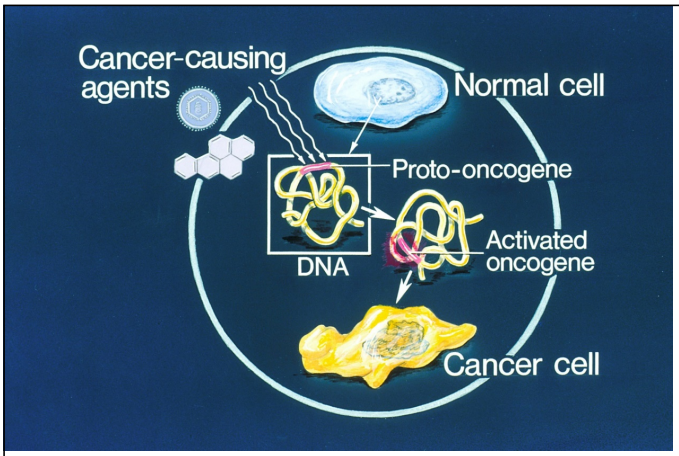
The New York Times 2002

TRIALS ARE HALTED ON A GENE THERAPY

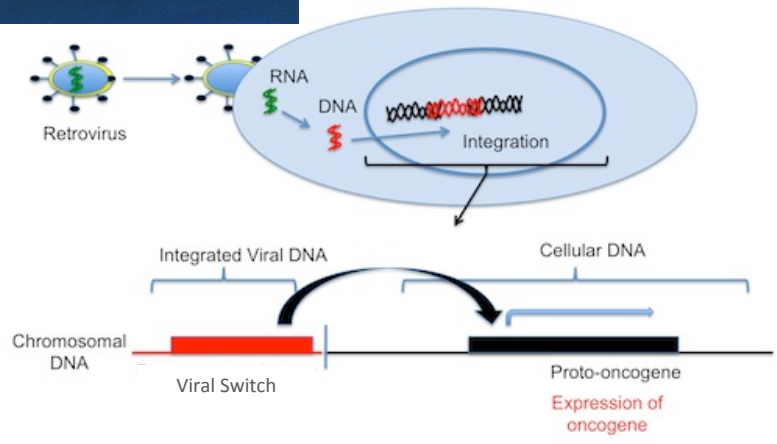
By SHERYL GAY STOLBERG
Published: October 4, 2002

WASHINGTON, Oct. 3— Officials in the United States and France said today that they had suspended four gene therapy experiments because the treatment, which cured a 3-year-old boy of a fatal immune deficiency, may have given him an illness similar to leukemia.

- 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



Retroviral Switches can Activate Proto-oncogenes and Induce Cancers



Some Early Problems with Human Gene Therapy

- Inefficient delivery of vector to target cells
- Low expression level 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

Science
AAAS

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

Forbes / Pharma & Healthcare

The Little Black Book of Billionaire Secrets

MAR 26, 2014 @ 06:00 AM 34,653 VIEWS

Gene Therapy's Big Comeback

Article

Phoenix rising: gene therapy makes a comeback. Acta Biochim Biophys Sin

Maria P Limberis

HEALTH

Gene Therapy Makes a Comeback With a Cautious, Supporting Role

By **MICHAEL WALDHOLZ** Staff Reporter of The Wall Street Journal
Updated May 30, 2002 12:01 a.m. ET

BloombergBusiness

Money Flows Again to Gene-Therapy Drugs Investors Once Shunned

Robert Langreth
May 20, 2015 — 4:00 AM PDT

Yang Lu

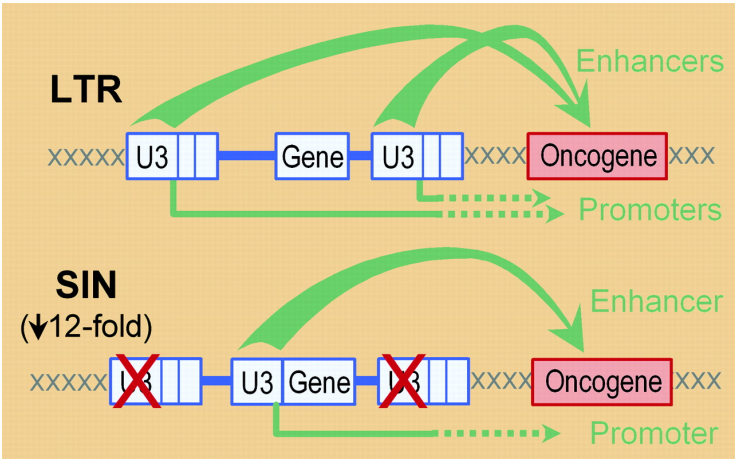
Gene therapy stages a comeback, albeit a humble one

Published on May 15, 2009

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

Development of Self-Inactivating (SIN) Vectors



1. First generation vectors often caused leukemia because they inserted viral DNA next to proto oncogenes (cancer causing genes).

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 are less likely to activate adjacent genes.

Self-Inactivating (SIN) Vectors are Effective in Gene Therapy

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Featured Research *from universities, journals, and other organizations*

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

Date: December 8, 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.

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"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".

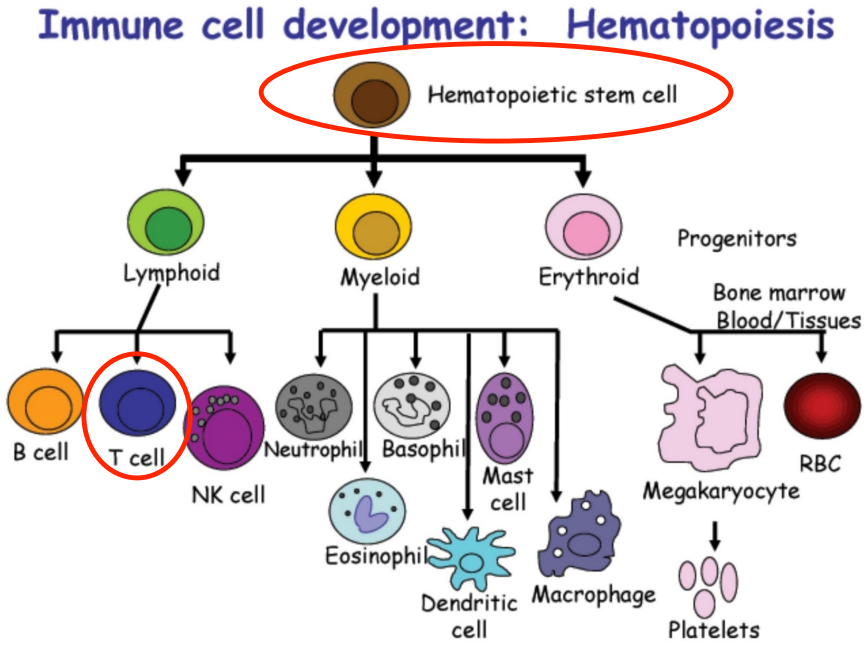
Fischer et al. 2015

Table 1. PID diseases and gene therapy

	First-generation γRV vectors	Second-generation SIN vectors	
	Effective	Effective	Planned
SCID X1	+[a]	+	
ADA deficiency	+	+	
WAS	+[b]	+	
SCID Rag-1			+
SCID Artemis			+
X-linked chronic granulomatous disease	+[b]		+
Leukocyte adhesion deficiency			+
HLH perforin deficiency			+[c]
HLH Munc13-4 deficiency			+[c]
XLP1			+[c]
IPEX (FoxP3 deficiency)			+[c]

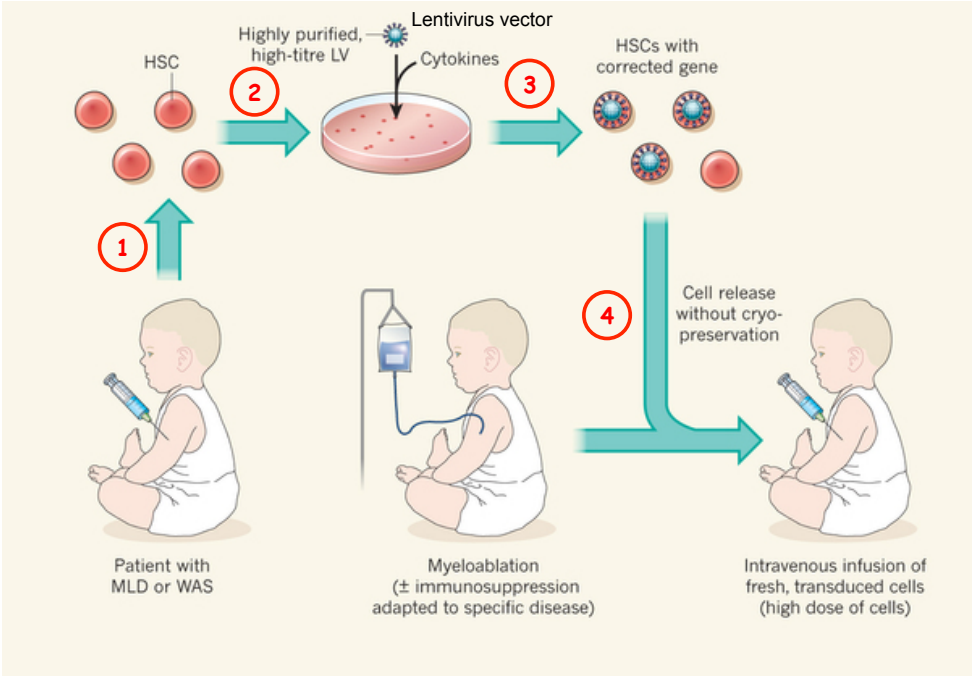
ADA, adenosine deaminase; HLH, hemophagocytic lymphohistiocytosis; IPEX, immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome; PID, primary immunodeficiencies; SAEs, serious adverse events; SCID, severe combined immunodeficiencies; SIN, self-inactivating; WAS, Wiskott–Aldrich syndrome.
[a]Associated with high frequency of SAEs (5 out of 19).
[b]Associated with very high frequency of SAEs (seven out of nine for WAS, and four out of four for CGD).
[c]CD34 and T cell strategy are both envisaged.

Using Stem Cells in *Ex Vivo* Gene Therapy



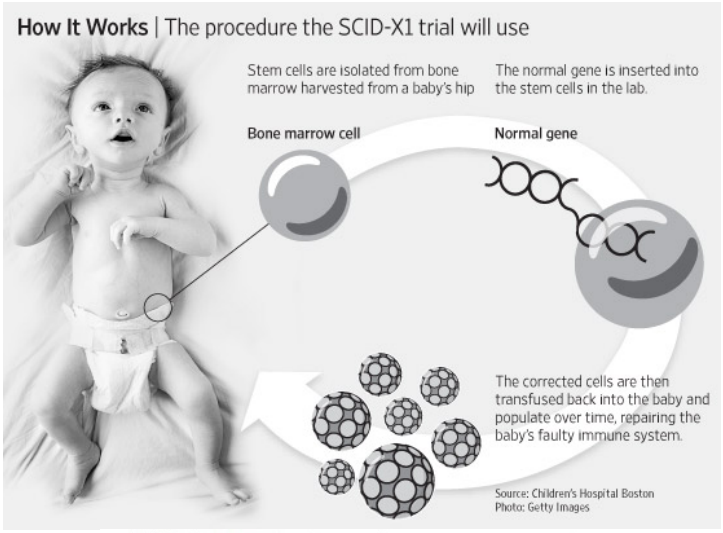
Dr. Pei-Yun Lee

General Strategy for Use of Hematopoietic Stem Cells in Gene Therapy



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



UCLA researcher pioneers gene therapy cure for 'Bubble Baby' disease

Game-changing stem cell treatment to be tested for sickle cell disease next

Peter Bracke | November 20, 2014

UCLA

It Works!

Gene therapy cures 'bubble boy disease'

31 Jan 2009, 1128 hrs IST, AP

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

Results after 10 years

- ADA-SCID – 4 of 6 children experienced immune reconstitution
- SCID-X1 – 9 of 10 children experienced normal T-cell number
- But - 5 of 20 SCID-X1 subjects experienced leukemia-like T lymphoproliferation in another study

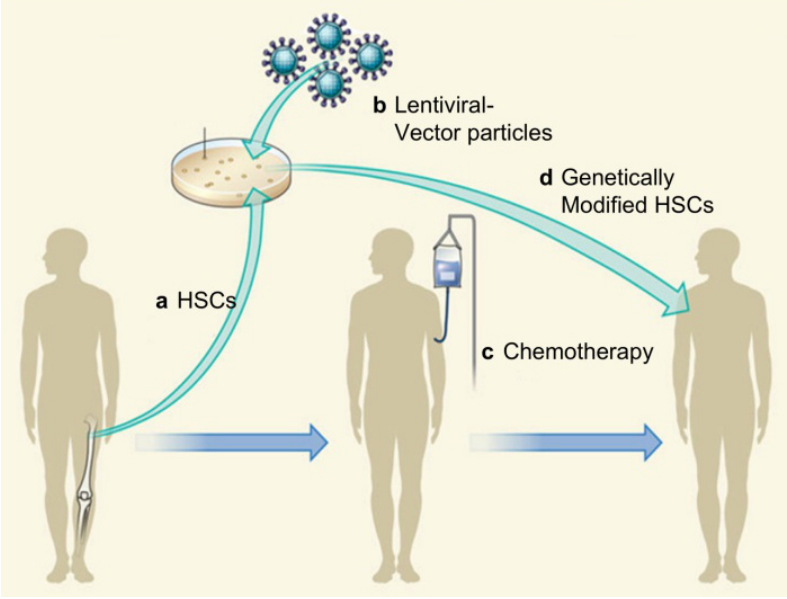
BUSINESS INSIDER

A gene therapy that cures a rare genetic disease just got its first customer, a year after it was approved

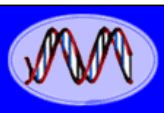
EMILY MULLIN, MIT TECHNOLOGY REVIEW
MAY 4, 2017, 11:57 AM

- ADA-SCID gene therapy product named Strimvelis from GlaxoSmithKline
- Approved for use in Europe
- One time treatment costs $665,000, with money back guarantee
- Cost of PEG-ADA treatment estimated at $60,000 per year in 1990 (FDA)

Other Diseases that are Being Targeted Using Ex Vivo Gene Therapy with Hematopoietic Stem Cells

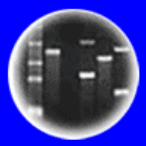


- ADA-SCID
- Chronic granulomatous disease
- Leucocyte adhesion deficiency
- SCID Artemis
- SCID Rag-1
- SCID-X1
- Sickle cell disease
- β-thalassaemia
- Wiskott Aldrich Syndrome
- X-linked lymphoproliferative syndrome



DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

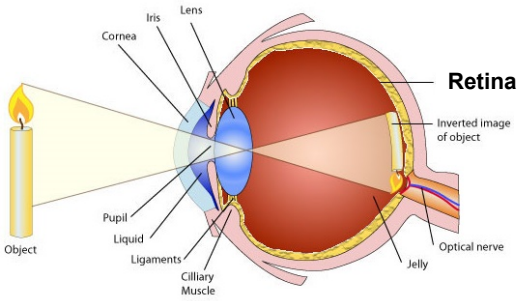
In Vivo Gene Therapy

Toolkit for In Vivo Somatic Cell Gene Therapy Procedures

1. Cloned copy of the therapeutic gene
2. Appropriate switch, often high expression level
3. Vector to transfer the gene into the cells
4. *Ability to target the vector to desired cells*

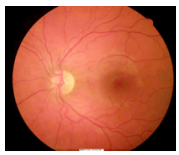
Blindness – Choroideremia (CHM)

How We See

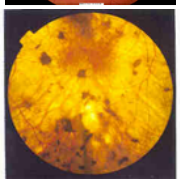


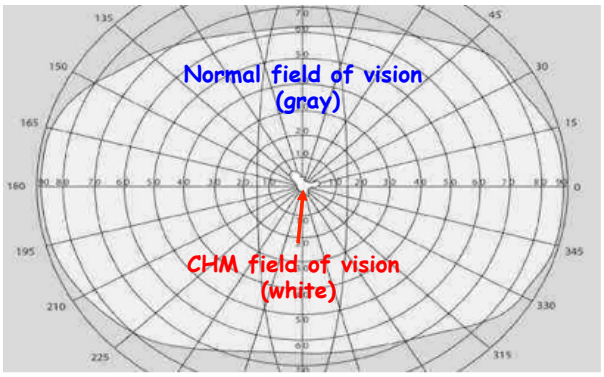
1. CHM is a rare inherited cause of blindness that affects around 1 in 50,000 people. Night blindness is an early symptom.
2. CHM is caused by mutation in the X-linked *REP1* (Rab escort protein) gene.
3. Without the REP1 protein, pigment cells in the retina die prematurely

Normal retina



CHM Retina





THE LANCET

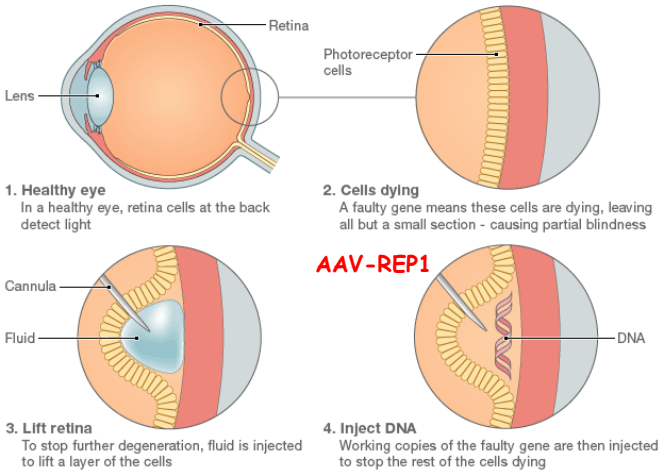
< Previous Article | Volume 383, No. 9923, p1129–1137, 29 March 2014 | Next Article >

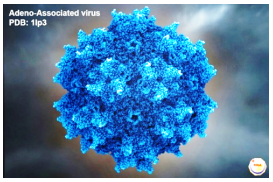
Articles

Retinal gene therapy in patients with choroideremia: initial findings from a phase 1/2 clinical trial

Prof Robert E MacLaren, FRCOphth, Markus Groppe, PhD, Alun R Barnard, PhD, Charles L Cottriall, PhD, Tanya Tolmachova, PhD, Prof Len Seymour, PhD, K Reed Clark, PhD, Prof Matthew J During, FACP, Prof Frans P M Cremers, PhD, Prof Graeme C M Black, FRCOphth, Prof Andrew J Lotery, FRCOphth, Susan M Downes, FRCOphth, Prof Andrew R Webster, FRCOphth, Prof Miguel C Seabra, MD

Gene therapy to prevent blindness





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

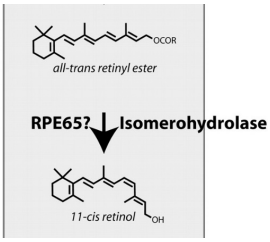
- @ 6 months (2013), vision improved in all 6 patients who received treatment
- @ 4 years (2017), 2 patients had significant vision improvement, 3 maintained their vision, 1 had slow decline in vision

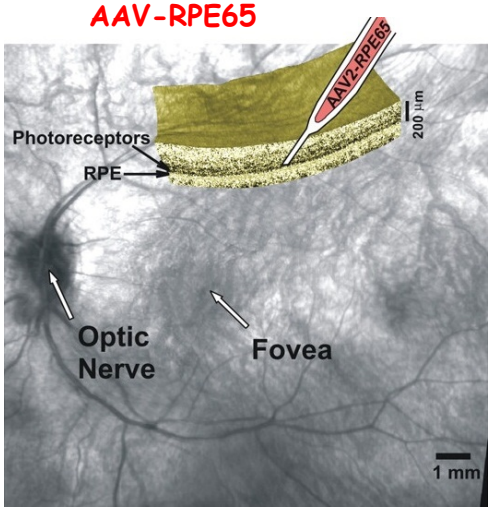
LCA Gene Therapy Using RPE65 & AAV2

Leber Congenital Amaurosis

- Degenerative diseases of the retina
- The most common cause of congenital blindness in children
- Gene therapy with AAV-RPE65

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





Cideciyan et al. PNAS 2008;105:15112

SUCCESS!

CAROLINE & COLE CARPER

The NEW ENGLAND JOURNAL of MEDICINE
May 14, 2015

BRIEF REPORT

Improvement and Decline in Vision with Gene Therapy in Childhood Blindness

Samuel G. Jacobson, M.D., Ph.D., Artur V. Cideciyan, Ph.D., Alejandro J. Roman, M.Sc., Alexander Sumaroka, Ph.D., Sharon B. Schwartz, M.S., C.G.C., Elise Heon, M.D., and William W. Hauswirth, Ph.D.

SUMMARY

Retinal gene therapy for Leber's congenital amaurosis, an autosomal recessive childhood blindness, has been widely considered to be safe and efficacious. Three years after therapy, improvement in vision was maintained, but the rate of loss of photoreceptors in the treated retina was the same as that in the untreated retina. Here we describe long-term follow-up data from three treated patients. Topographic maps of visual sensitivity in treated regions, nearly 6 years after therapy for two of the patients and 4.5 years after therapy for the third patient, indicate progressive diminution of the areas of improved vision. (Funded by the National Eye Institute; ClinicalTrials.gov number, NCT00481546.)

The New York Times | http://nyti.ms/1OebWaX

SCIENCE

Eye Treatment Closes In on Being First Gene Therapy Approved in U.S.

By ANDREW POLLACK OCT. 5, 2015

What could become the first gene therapy to win approval in the United States moved closer to market on Monday, when its developer announced that the medicine had succeeded in a late-stage clinical trial in treating an inherited eye disease that can cause blindness.

Rewriting Life

Gene Therapy in U.S. Is On Track for Approval as Early as Next Year

Spark Therapeutics is within striking distance of a landmark green light from the FDA for its treatment for certain forms of blindness.

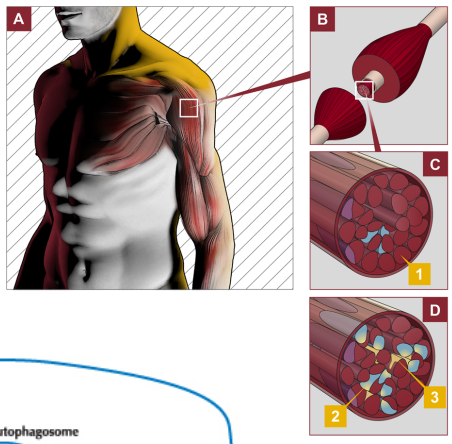
by Emily Mullin October 18, 2016

The first gene therapy for an inherited disease in the U.S. is closer to reality than ever before.

Spark Therapeutics is only the second company to pursue an application to the U.S. Food and Drug Administration for such a treatment, but it's likely to be the first to hit the market.

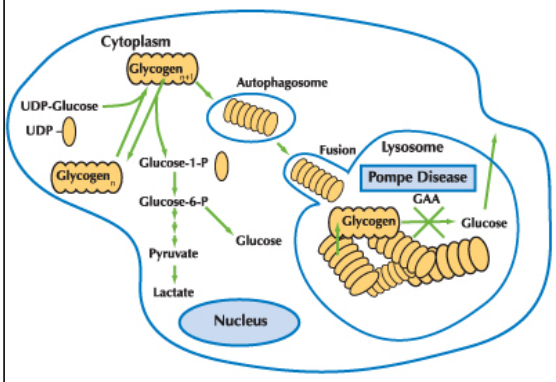
Speaking at EmTech MIT 2016 on Tuesday, Katherine High, Spark's cofounder, confirmed that the company is on track to launch its first product next year. The gene therapy, known as SPK-RPE65, targets mutations in people's eyes that often lead to blindness. Currently, there are no drugs available to treat these disorders, known as inherited retinal dystrophies.

Pompe Disease



- **A** Skeletal muscle
- **B** Bundle of muscle fibers
- **C** Normal breakdown of glycogen by GAA in muscle cells
- **D** Harmful build-up of glycogen in the muscle cells due to lack of GAA

1. Glycogen is broken down in parts of each cell called lysosomes
2. In Pompe disease, glycogen builds up in the lysosomes, damaging the muscle cells
3. As the condition worsens, glycogen leaks out of the lysosomes, damaging the surrounding muscle cells and weakening the muscle



- **Glycogen is stored in the lysosome, an organelle**
- **Acid alpha glucosidase (GAA) converts glycogen, a storage form of sugar, into glucose**
- **GAA is defective in individuals with Pompe disease. Glycogen overaccumulation damages muscle cells.**
- **Pompe disease occurs in 1 in 40,000 births**

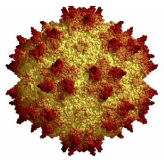
Gene Therapy for Pompe Disease With AAV-GAA

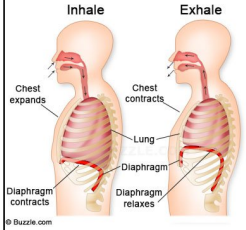
HUMAN GENE THERAPY 24:630–640 (June 2013)

DOI: 10.1089/hum.2012.250

Phase I/II Trial of Adeno-Associated Virus–Mediated Alpha-Glucosidase Gene Therapy to the Diaphragm for Chronic Respiratory Failure in Pompe Disease: Initial Safety and Ventilatory Outcomes

Barbara K. Smith,[1] Shelley W. Collins,[2] Thomas J. Conlon,[2,3] Cathryn S. Mah,[2,3] Lee Ann Lawson,[2] Anatole D. Martin,[1] David D. Fuller,[1] Brian D. Cleaver,[2,3] Nathalie Clément,[2,3] Dawn Phillips,[2] Saleem Islam,[2,4] Nicole Dobjia,[5] and Barry J. Byrne[2,3]





Approach

- Used AAV-GAA
- Targeted the diaphragm to aid in breathing

Results

- Improved the volume of air displace during breathing
- Periods of unassisted breathing increased
- No adverse immune responses detected

Experimental Neurology 287 (2017) 216–224

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Research Paper

Inspiratory muscle conditioning exercise and diaphragm gene therapy in Pompe disease: Clinical evidence of respiratory plasticity

Barbara K. Smith [a,b,*], A. Daniel Martin [a], Lee Ann Lawson [b], Valerie Vernot [c], Jordan Marcus [e], Nadeem Shafi [f], Manuela Corti [b], Shelley W. Collins [b], Barry J. Byrne [b]

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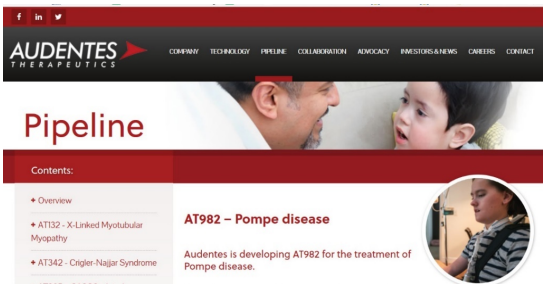
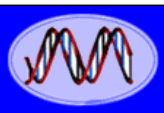
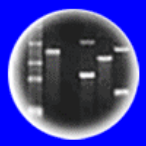
[f] Department of Pediatrics Critical Care Division, University of Tennessee Health Science Center, 50 N. Dunlap, Memphis, TN 38103, United States





DNA
Genetic Code of Life

Entire Genetic Code of a Bacteria



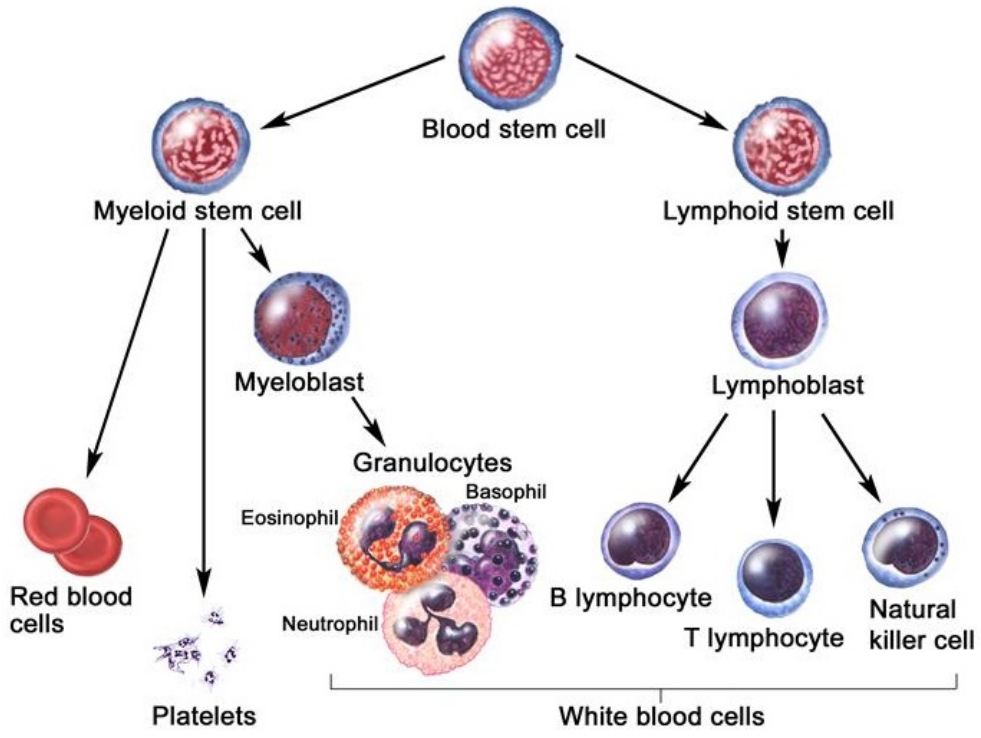
DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

Gene Therapy to Control Cancers

Normal Blood Cell Development



The New York Times

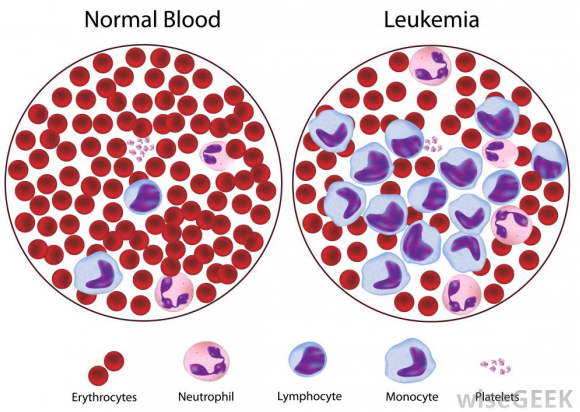
December 9, 2012

2013

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

By DENISE GRADY

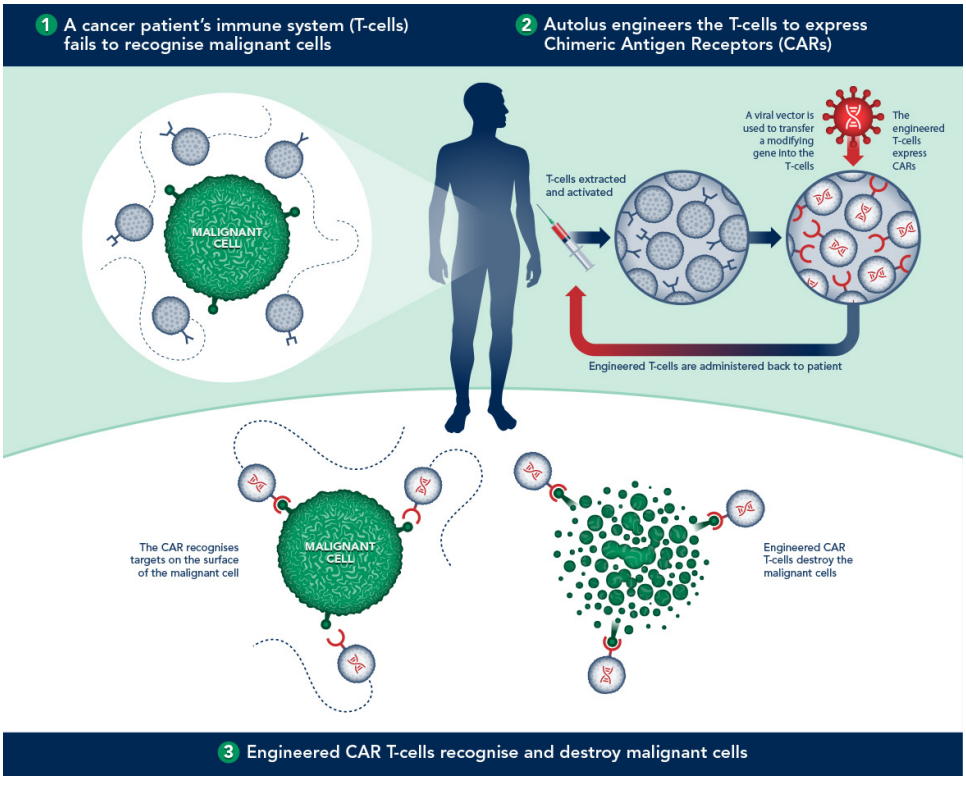
Leukemia is cancer of the blood, that results in an increase in immature white blood cells. Chronic lymphoid leukemia affects B cell lymphocytes



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

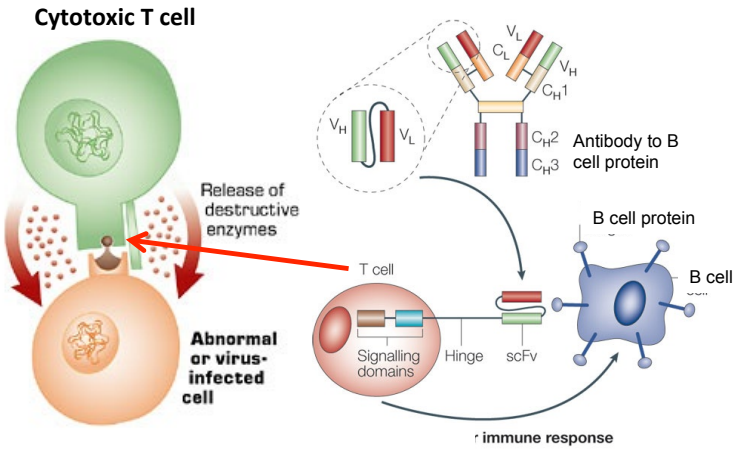
Emily Whitehead, alive at age 7 because of a novel gene therapy strategy

Chimeric Antigen Receptor (CAR) Strategy



Ex-vivo Gene Therapy for Lymphocytic Leukemia

Science Translational Medicine AAAS

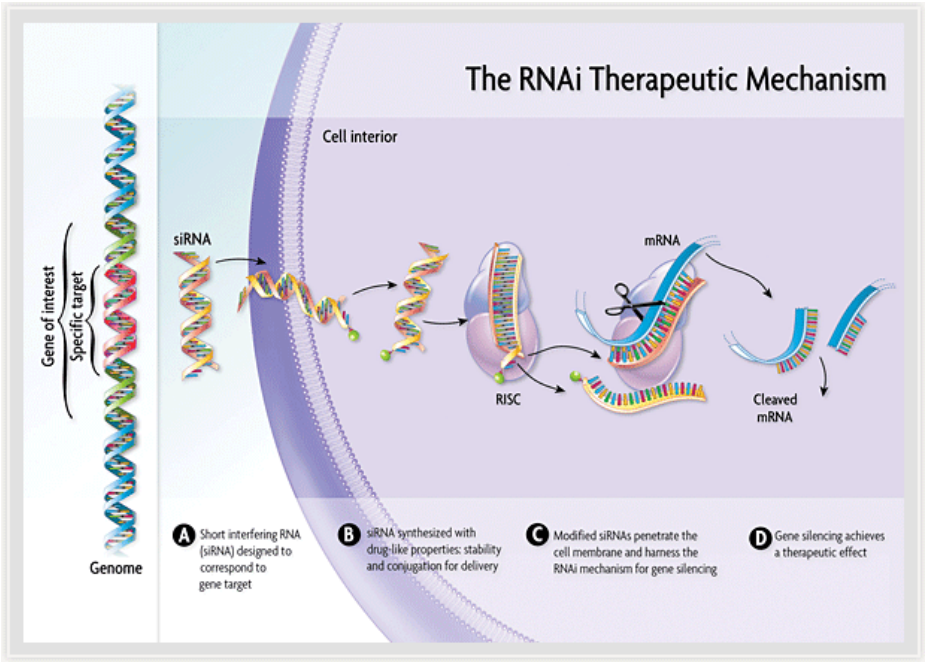


Protocol

- Removed T cells from patients
- Created gene encoding Chimeric Antigen Receptor (CAR) that recognize a protein on the surface of B cells
- Transferred CAR gene 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

RNA Interference
Silencing Genes Through mRNA Degradation



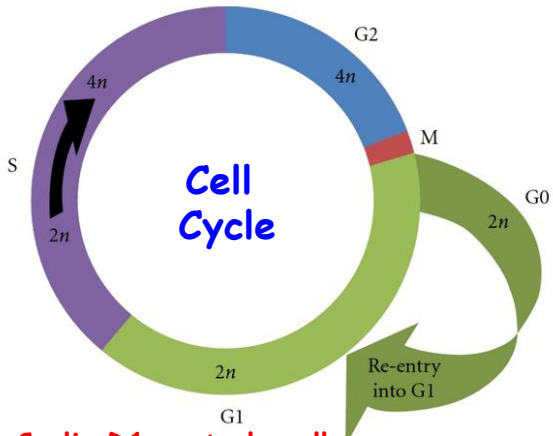
DNA → ~~mRNA~~ → ~~Protein~~

Silencing Genes to Control Cancer

Mantle Cell Lymphoma is a Cancer of Leukocytes Characterized by Overproduction of Cyclin D1 – A Cell Cycle Regulator

Harnessing RNAi-based nanomedicines for therapeutic gene silencing in B-cell malignancies 2016

Shiri Weinstein[a,b,c,1], Itai A. Toker[a,b,c,1], Rafi Emmanuel[a,b,c], Srinivas Ramishetti[a,b,c], Inbal Hazan-Halevy[a,b,c], Daniel Rosenblum[a,b,c], Meir Goldsmith[a,b,c], Avigdor Abraham[d], Ohad Benjamini[d], Osnat Bairey[e], Pia Raanani[e], Arnon Nagler[d], Judy Lieberman[f,g], and Dan Peer[a,b,c,2]



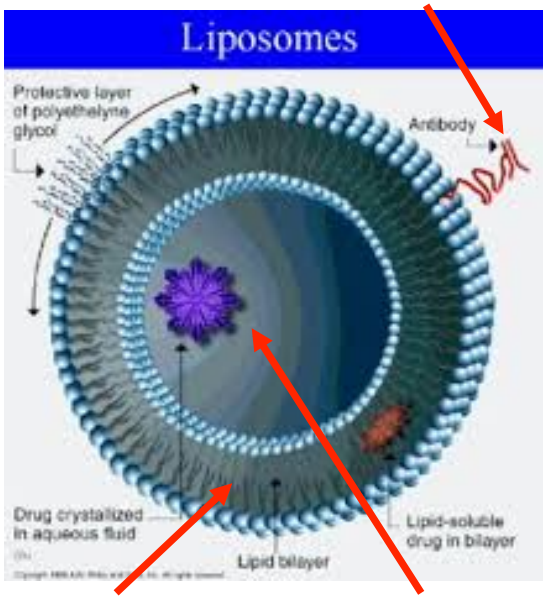
Cyclin D1 controls cell entry into DNA synthesis

Gene Therapy Approach

- Designed small interfering RNAs that would silence the cyclin D1 gene through RNA interference
- Targeted the siRNAs specifically to malignant leukocytes using a lipid nanoparticle
- Delivery of siRNA to MCL cells inhibited cyclin D1 and prolonged survival of tumor-bearing mice

Lipid-based Nanoparticle for Specific Delivery of siRNA to MCL Cells *in Vivo*

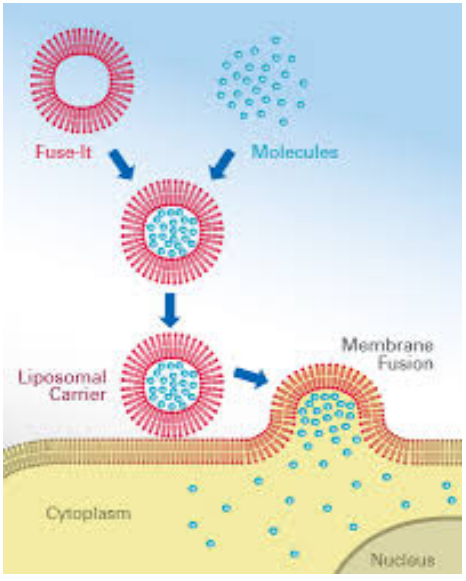
Antibody that binds MCL cells

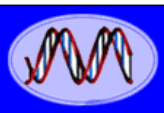


Lipid bilayer similar to cell membrane

siRNAs are sequested in liposome

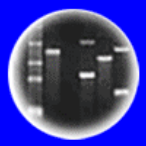
Delivery of siRNAs to MCL cell





DNA
Genetic Code of Life

Entire Genetic Code of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

Regulation of Gene Therapy

US Regulatory Authority for Gene Therapy

- Department of Health and Human Services (DHHS) has been charged with oversight of clinical trials
  - 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

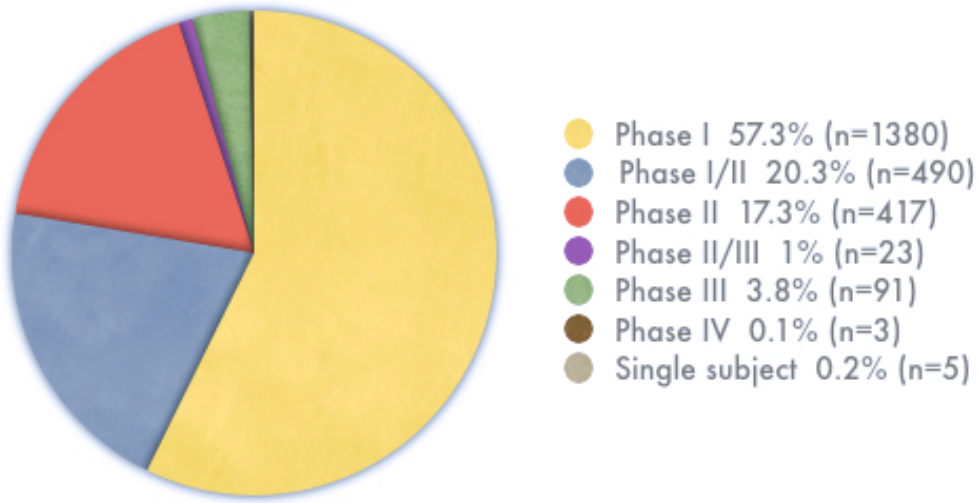
Clinical Trials

Phase I	Phase II	Phase III	Phase IV
20-80 participants	**100-300 participants**	**1,000-3,000 participants**	**Thousands of participants**
Up to several months	Up to (2) years	One (1) - Four (4) years	One (1) year +
Studies the safety of medication/treatment	Studies the efficacy	Studies the safety, efficacy and dosing	Studies the long-term effectiveness; cost effectiveness
70% success rate	33% success rate	25-30% success rate	70-90% success rate

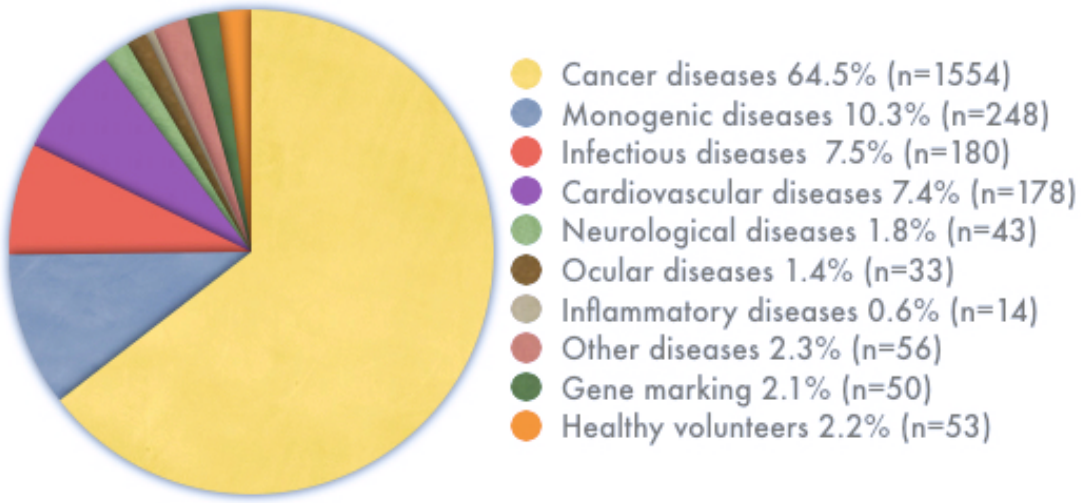
COMPARISON OF CLINICAL TRIAL PHASES

	PHASE I	PHASE II	PHASE III	PHASE IV
OBJECTIVES:	Determine the metabolic and pharmacological actions and the maximally tolerated dose	Evaluate effectiveness, determine the short-term side effects and identify common risks for a specific population and disease	Obtain additional information about the effectiveness on clinical outcomes and evaluate the overall risk-benefit ratio in a demographically diverse sample	Monitor ongoing safety in large populations and identify additional uses of the agent that might be approved by the FDA
FACTORS TO BE IDENTIFIED:	-Bioavailability -Bioequivalence -Dose proportionality -Metabolism -Pharmacodynamics -Pharmacokinetics	-Bioavailability -Drug-disease interactions -Drug-drug interactions -Efficacy at various doses -Pharmakodynamics -Pharmakokinetics -Patient safety	-Drug-disease interactions -Drug-drug interactions -Dosage intervals -Risk-benefit information -Efficacy and safety for subgroups	-Epidemiological data -Efficacy and safety within large, diverse populations -Pharmacoeconomics
DATA FOCUS:	-Vital signs -Plasma and serum levels -Adverse events	-Dose response and tolerance -Adverse events -Efficacy	-Laboratory data -Efficacy -Adverse events	-Efficacy -Pharmacoeconomics -Epidemiology -Adverse events
DESIGN FEATURES:	-Single, ascending dose tiers -Unblinded -Uncontrolled	-Placebo controlled comparisons -Active controlled comparisons -Well-defined entry criteria	-Randomized -Controlled -2-3 treatment arms -Broader eligibility criteria	-Uncontrolled -Observational
DURATION:	Up to 1 month	Several months	Several years	Ongoing (following FDA approval)
POPULATION:	Healthy volunteers or individuals with the target disease (such as cancer or HIV)	Individuals with target disease	Individuals with target disease	Individuals with target disease, as well as new age groups, genders, etc.
SAMPLE SIZE:	20 to 80	200 to 300	Hundreds to thousands	Thousands
EXAMPLE:	Study of a single dose of Drug X in normal subjects	Double-blind study evaluating safety and efficacy of Drug X vs. placebo in patients with hypertension	Study of Drug X vs. standard treatment in hypertension study	Study of economic benefit of newly-approved Drug X vs. standard treatment for hypertension

Phases of Gene Therapy Clinical Trials



Indications Addressed by Gene Therapy Clinical Trials



Approved Gene Therapy Products Worldwide

No gene therapy products have been approved for use in the United States

P53 tumor suppressor deficiency
Marketed in China 2004

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.

GlaxoSmithKline
Consumer Healthcare

Strimvelis®
ADA-SCID
Marketed in Europe 2016
EMA APPROVED

Lipoprotein lipase deficiency
Marketed in Europe 2012

Glybera is a gene therapy that is designed to restore the LPL enzyme activity required to enable the processing, or clearance, of fat-carrying 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.

April 20, 2017

UniQure Says It Will Not Pursue EC Marketing Renewal for Glybera Gene Therapy

The World's Most Expensive Medicine Is a Bust

The first gene therapy approved in the Western world costs $1 million and has been used just once. The doctor who tried it says the price is "absolutely too high."

by Antonio Regalado May 4, 2016

Issues Concerning Gene Therapy

Gene Therapy for Human Genetic Disease?

Proposals for genetic manipulation in humans raise difficult scientific and ethical problems.

Theodore Friedmann and Richard Roblin

3 March 1972, Volume 175, Number 4025 SCIENCE

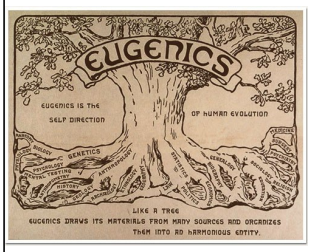
We propose the following ethico-scientific criteria which any prospective techniques for gene therapy in human patients should satisfy:

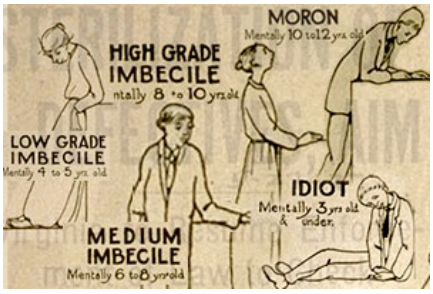
1. There should be adequate biochemical characterization of the prospective patient's genetic disorder.
2. There should be prior experience with untreated cases of what appears to be the same genetic defect
3. There must be an adequate characterization of the quality of the exogenous DNA vector.
4. There 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

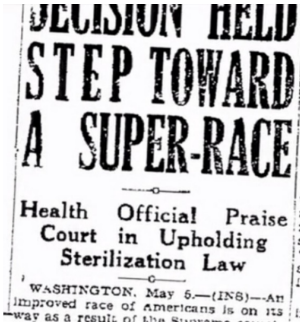
Some Issues With Human Gene Therapy

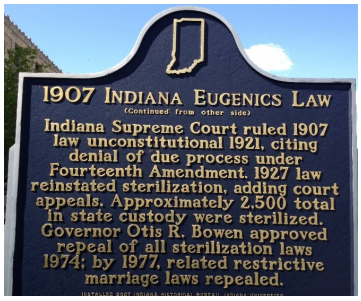
- Risks
- Enhancement
- Consent
- Availability To Everyone
- Eugenics (Germ Line)

Eugenics: The study of or belief in the possibility of improving the qualities of the human species or a human population, especially by such means as discouraging reproduction by persons having genetic defects or presumed to have inheritable undesirable traits (negative eugenics) or encouraging reproduction by persons presumed to have inheritable desirable traits (positive eugenics) – dictionary.com



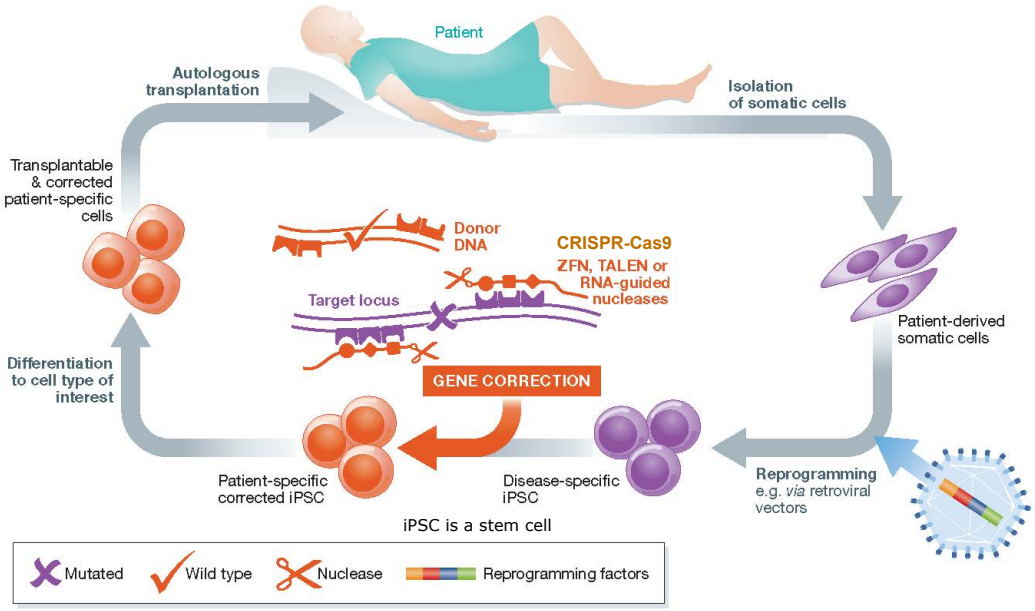






Gene Editing & Human Gene Therapy

Human Genome Editing Therapy

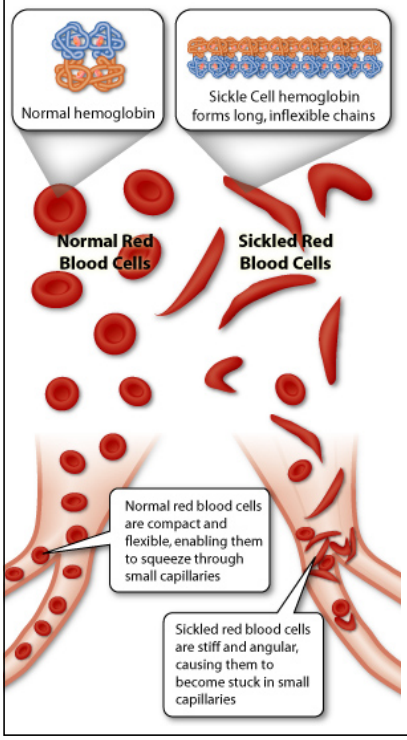


Uses of Genome Editing

- Correct monogenic disorders – germline and somatic cells
  - Induce precise sequence changes to correct mutations
- Engineering pathogen DNA to combat infectious disease
  - Mutate integrated proviral DNA in host cells
- Induce therapeutic or protective mutations
  - Introduce mutations that cause resistance to HIV infection

Xiao-Jie et al. 2015

Sickle Cell Disease



ScienceDaily

Your source for the latest research news

Step toward gene therapy for sickle cell disease

Date: November 8, 2016

Source: Stanford University Medical Center

Summary: A gene-editing tool known as CRISPR has been used to repair the gene that causes sickle cell disease in human stem cells, which they say is a key step toward developing a gene therapy for the disorder.

Gene Therapy

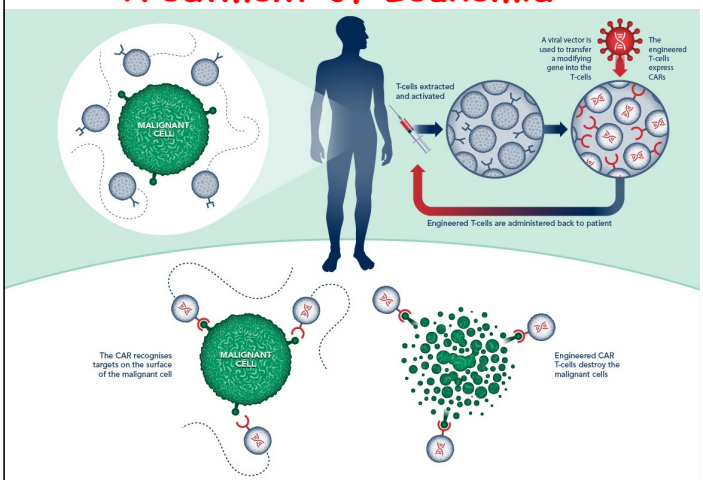
- CRISPR-Cas9 was used to correct the β-hemoglobin gene
- Experiments were done in human hematopoietic stem cells
- Procedure potentially can be used to treat β-hemoglobinopathies, such as sickle cell disease and β-thalassaemia

nature International weekly journal of science

Targeting a CAR to the *TRAC* locus with CRISPR/Cas9 enhances tumour rejection

Justin Eyquem, Jorge Mansilla-Soto, Theodoros Giavridis, Sjoukje J. C. van der Stegen, Mohamad Hamieh, Kristen M. Cunanan, Ashlesha Odak, Mithat Gönen & Michel Sadelain

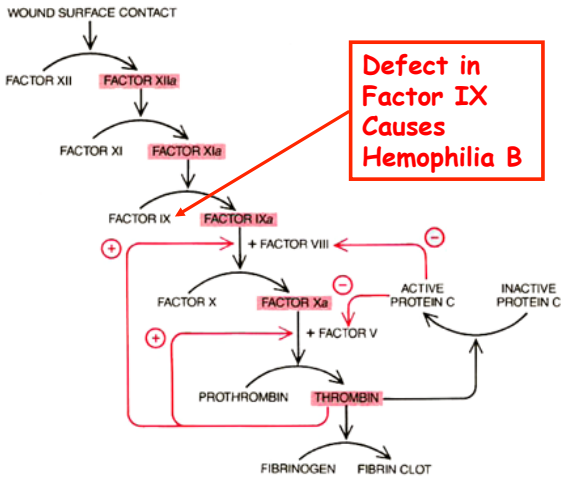
CAR T Cells Treatment of Leukemia



Gene Therapy

- Used CRISPR-Cas9 to insert a CD19-specific CAR to the T-cell receptor a constant (TRAC) locus.
- Enhanced T-cell potency, with edited cells vastly outperforming conventionally generated CAR T cells
- Studies done in a mouse model of acute lymphoblastic leukemia

Gene Therapy for Hemophilia Using CRISPR-Cas9



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.

ScienceDaily

Your source for the latest research news

CRISPR used for first time to correct clotting in newborn and adult mice

Date: November 30, 2016

Source: University of Pennsylvania School of Medicine

Gene Therapy

- Used CRISPR-Cas9 to correct a defect in the Clotting Factor IX gene
- Used an AAV vector to target CRISPR-Cas9 to liver cells in a mouse Hemophilia B model
- Mice showed stable Factor IX activity at or above normal levels over four months

The Future is Now for Human Genome Editing Therapy

TRANSGENIC ANIMALS

Editing of Targeted Genes Proved Possible in Monkeys

Stem Cells. 2015 May;33(5):1470-9. doi: 10.1002/stem.1969.

Production of Gene-Corrected Adult Beta Globin Protein in Human Erythrocytes Differentiated from Patient iPSCs After Genome Editing of the Sickle Point Mutation.

Huang X[1], Wang Y, Yan W, Smith C, Ye Z, Wang J, Gao Y, Mendelsohn L, Cheng L.

Genome Res. 2014 Sep;24(9):1526-33. doi: 10.1101/gr.173427.114. Epub 2014 Aug 5.

Seamless gene correction of β-thalassemia mutations in patient-specific iPSCs using CRISPR/Cas9 and piggyBac.

Xie F[1], Ye L[1], Chang JC[1], Beyer AI[2], Wang J[3], Muench MO[4], Kan YW[5].

World first use of gene-edited immune cells to treat 'incurable' leukaemia

05 November 2015

< Previous Article | Volume 13, Issue 6, p659–662, 5 December 2013

Brief Report | Switch to Standard

Correction of a Genetic Disease in Mouse via Use of CRISPR-Cas9

Yuxuan Wu[7], Dan Liang[7], Yinghua Wang, Meizhu Bai, Wei Tang, Shiming Bao, Zhiqiang Yan, Dangsheng Li, Jinsong Li

[7] These authors contributed equally to this work

< Previous Article | Volume 13, Issue 6, p653–658, 5 December 2013

Brief Report | Switch to Standard

Functional Repair of CFTR by CRISPR/Cas9 in Intestinal Stem Cell Organoids of Cystic Fibrosis Patients

Gerald Schwank[7], Bon-Kyoung Koo[7,8], Valentina Sasselli, Johanna F. Dekkers, Inha Heo, Turan Demircan, Nobuo Sasaki, Sander Boymans, Edwin Cuppen, Cornelis K. van der Ent, Edward E.S. Nieuwenhuis, Jeffrey M. Beekman, Hans Clevers

[7] These authors contributed equally to this work

[8] Present address: Wellcome Trust Medical Research Council Stem Cell Institute, University of Cambridge, Cambridge CB2 1QR, UK

Prevention of muscular dystrophy in mice by CRISPR/Cas9–mediated editing of germline DNA

Chengzu Long[1,*], John R. McAnally[1,*], John M. Shelton[2], Alex A. Mireault[1], Rhonda Bassel-Duby[1], Eric N. Olson[1,†]

+ Author Affiliations

†To whom correspondence should be addressed. E-mail: eric.olson@utsouthwestern.edu

*These authors contributed equally to this work.

Science 05 Sep 2014:
Vol. 345, Issue 6201, pp. 1184-1188
DOI: 10.1126/science.1254445

Editing the genome to introduce a beneficial naturally occurring mutation associated with increased fetal globin

Beeke Wienert, Alister P. W. Funnell, Laura J. Norton, Richard C. M. Pearson, Lorna E. Wilkinson-White, Krystal Lester, Jim Vadolas, Matthew H. Porteus, Jacqueline M. Matthews, Kate G. R. Quinlan & Merlin Crossley

Nature Communications **6**, Article number: 7085 doi:10.1038/ncomms8085

Received 22 September 2014 Accepted 31 March 2015 Published 14 May 2015

What About Using CRISPR-Cas9 for Human Gene Therapy?

nature International weekly journal of science

CRISPR gene-editing tested in a person for the first time

The move by Chinese scientists could spark a biomedical duel between China and the United States.

David Cyranoski

15 November 2016

Using CRISPR-Cas9 to correct a defect in PD-1, which normally puts the brakes on a cell's immune response

First CRISPR clinical trial gets green light from US panel

The technique's first test in people could begin as early as the end of the year.

Sara Reardon

22 June 2016

Using CRISPR-Cas9 to help augment cancer therapies that rely on enlisting a patient's T cells

Commercialization of CRISPR/CAS9 for Gene Therapy

CRISPR THERAPEUTICS

Intellia THERAPEUTICS

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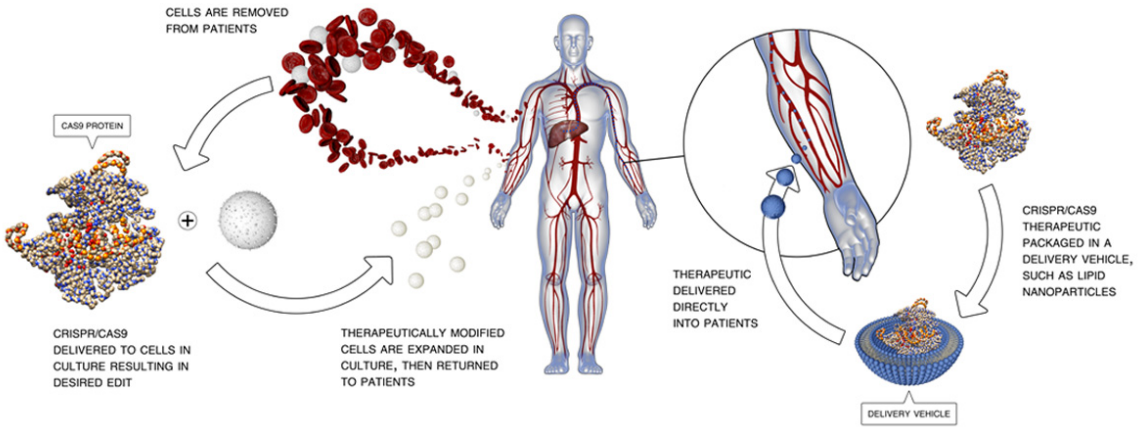
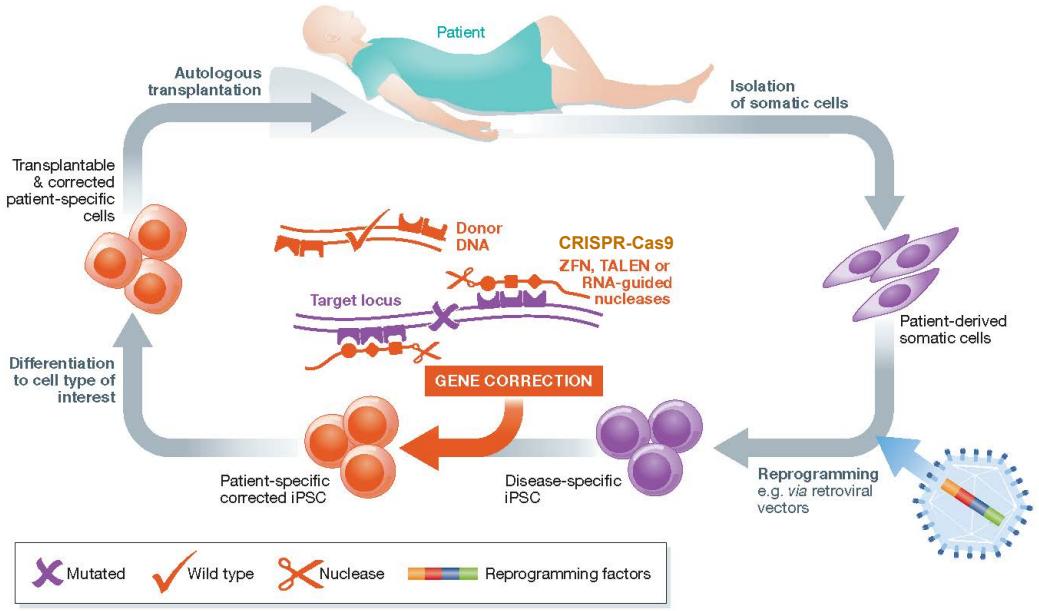
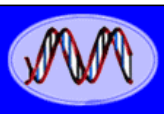
Ex Vivo

In Vivo



Human Genome Editing Therapy





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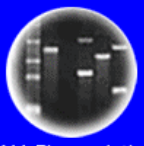

DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

The End!!

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