HC70A, SAS70A, & PLSS059
Winter 2018
Genetic Engineering in Medicine, Agriculture, and Law

Professors Bob Goldberg, John Harada, & Channapatna Prakash

Lecture 5
Human Genetic Engineering and Gene Therapy

UCLA TUSKEGEE UNIVERSITY UCDAVIS UNIVERSITY OF CALIFORNIA

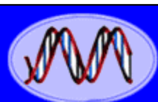
THEMES

Human Genetic Engineering and Gene Therapy

1. What is Gene Therapy?
2. Case Study of Gene Therapy for Severe Combined Immunodeficiency (SCID)
    a. Types of Gene Therapy
    b. Vectors
    c. Some Problems and Improvements with Gene Therapy
    d. Regulation of Gene Therapy
3. Other Ex Vivo Gene Therapies
4. *In Vivo* Gene Therapies
5. Current Status of Gene Therapy
6. Issues Concerning Gene Therapy
7. Gene Editing & Human Gene Therapy

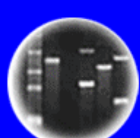
Genetically Engineered Organisms & Their Uses

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



DNA
Genetic Code of Life

Entire Genetic Code of a Bacteria

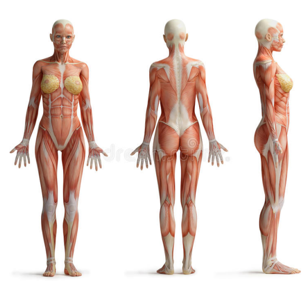


DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

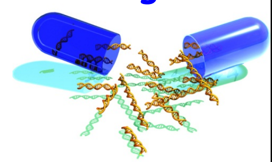
Plants of Tomorrow

Human Genetic Engineering and Gene Therapy

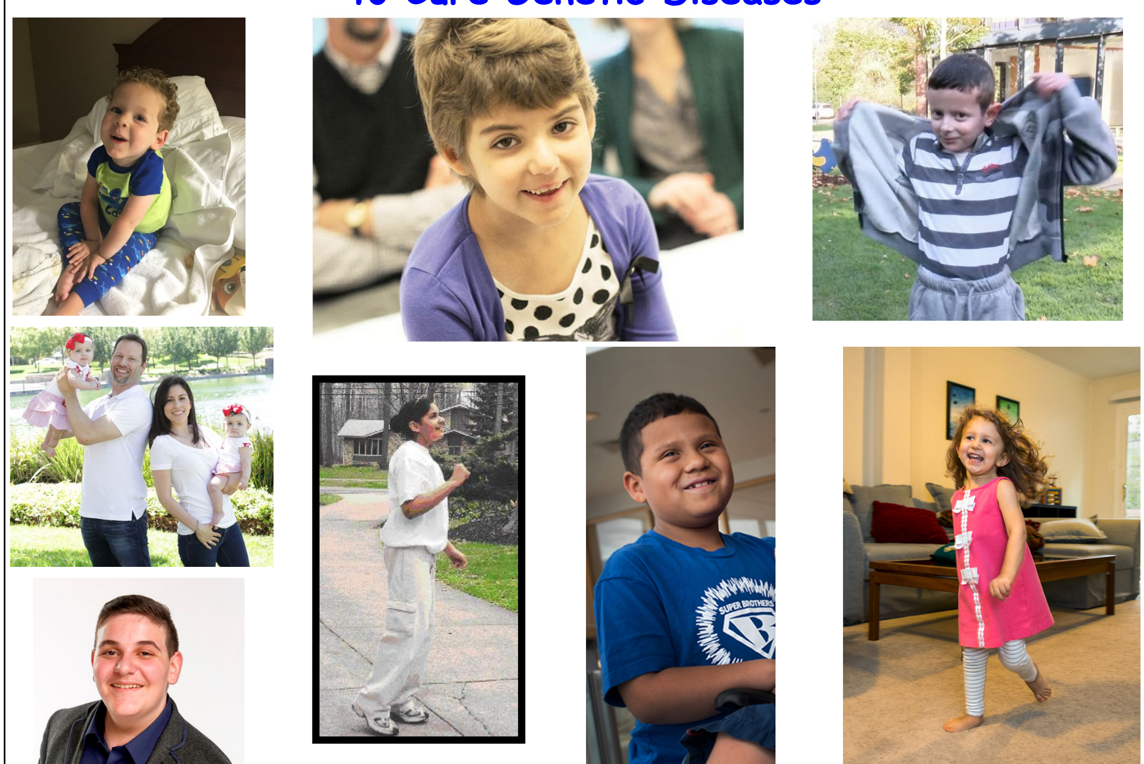


What is Gene Therapy?

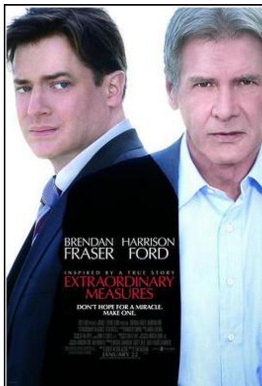
- The insertion of usually genetically altered genes into cells especially to replace defective genes in the treatment of genetic disorders or to provide a specialized disease-fighting function - *Merriam-Webster Dictionary*
- Experimental treatment of a genetic disorder by replacing, supplementing, 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*

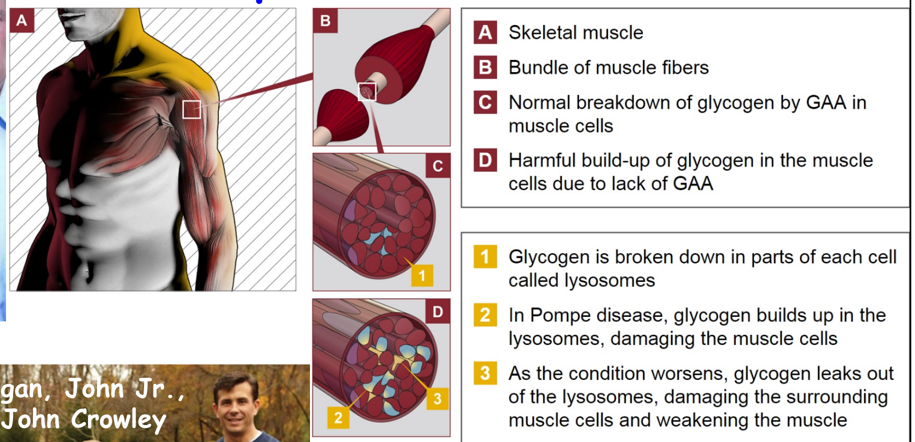


Humans Have Been Genetically Engineered to Cure Genetic Diseases



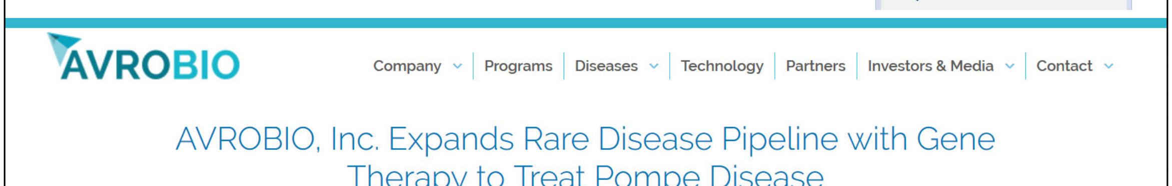
Pompe Disease

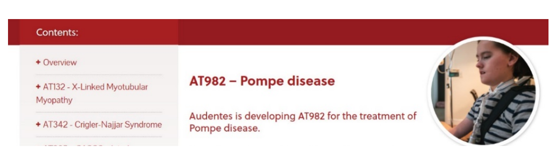




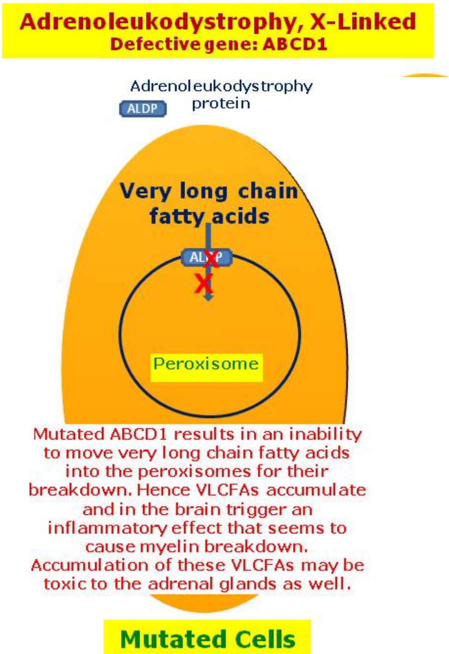
- Glycogen is stored in the lysosome, an organelle
- Acid alpha glucosidase (GAA) converts glycogen, a storage form of sugar, into glucose
- GAA is defective in individuals with Pompe disease. Glycogen overaccumulation damages muscle cells.
- Pompe disease occurs in 1 in 40,000 births

Gene Therapy for Pompe Disease With the Acid Alpha Glucosidase Gene

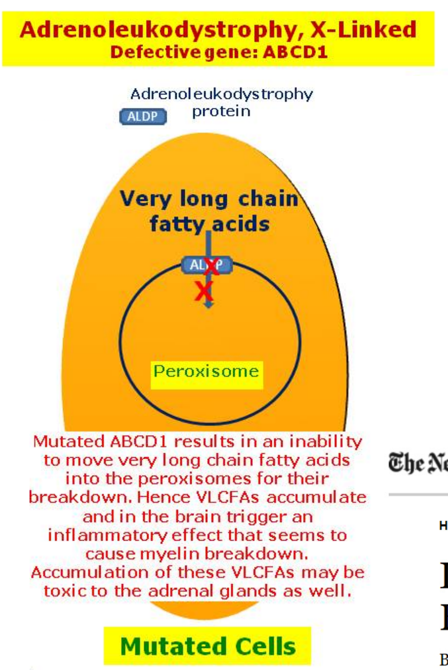




Adrenoleukodystrophy (ALD)



Gene Therapy for ALD Disease



The New York Times | https://nyti.ms/2xWXIZc

HEALTH

In a First, Gene Therapy Halts a Fatal Brain Disease

By GINA KOLATA OCT. 5, 2017

For the first time, doctors have used gene therapy to stave off a fatal degenerative brain disease, an achievement that some experts had thought impossible.

Hemophiliacs Have Mutations in Factor VIII, Factor IX, or Factor XI Genes

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

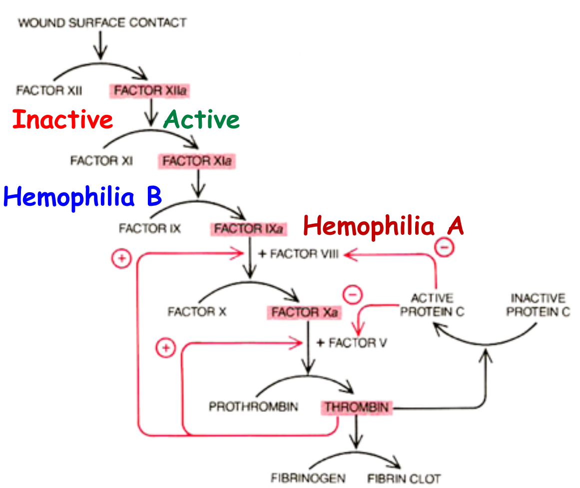
18,000 People in US Have Hemophilia & 400 Babies/Year Are Born With Disorder
Prior to 1960s – Average Life Span Was 11 Years

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

Hypothesis For High Frequency in Males?

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

Protein Factors in Blood Lead To Clotting



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.

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!

Gene Therapies for Hemophilia A & B

The Guardian
US edition

Sarah Boseley *Health editor*
Wed 13 Dec 2017 19.01 EST

Leap forward towards gene therapy cure for haemophilia A

Scientists around the world congratulate the team that has made a major advance in finding a cure for the life-threatening blood disorder

The Washington Post
Democracy Dies in Darkness

Gene therapy makes a big advance treating hemophilia B blood disorder

By **Laurie McGinley** December 6, 2017 Email the author

Sangamo THERAPEUTICS

About | Technology | Product Pipeline | Patients + Families | Clinical Trials | Collaborations | Investors + Media | Careers

Overview | Hemophilia | Lysosomal Storage Disorders | Hemoglobinopathies | Central Nervous System | HIV

Lead Indication	Approach	Program	Research	Preclinical	Phase 1/2	Phase 3
Hemophilia A		Pfizer	■	■	■	
Hemophilia B		SB-FIX	■	■	■	

The Future of Human Gene Therapy is Now!

Bluebird Bio Presents Positive Early Results of Gene Therapy LentiGlobin for Sickle Cell Disease

JANUARY 9, 2018 BY PATRICIA INACIO, PHD

Science News *from research organizations*

Gene therapy improves immunity in babies with 'bubble boy' disease

Date: December 10, 2017
Source: St. Jude Children's Research Hospital
Summary: Preliminary findings indicate that new gene therapy is safe and effective for babies with a devastating inherited disorder that leaves them with little or no immune protection.

December 9, 2012

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

By DENISE GRADY

Science Home News Journals Topics Careers

Gene therapy's new hope: A neuron-targeting virus is saving infant lives

By **Jocelyn Kaiser** | Nov. 1, 2017 , 5:00 PM

HEALTH

Gene Therapy Creates Replacement Skin to Save a Dying Boy

Leer en español

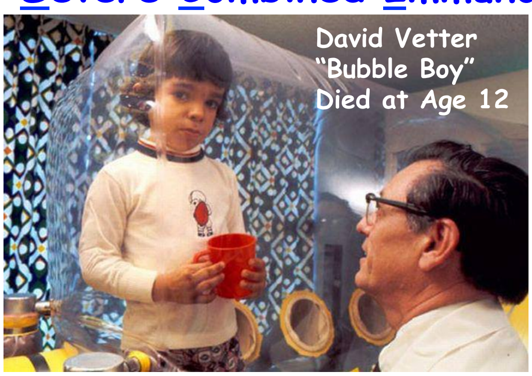
By DENISE GRADY NOV. 8, 2017

Modified T cells that attack leukemia become first gene therapy approved in the United States

By **Jocelyn Kaiser** | Aug. 30, 2017 , 2:48 PM

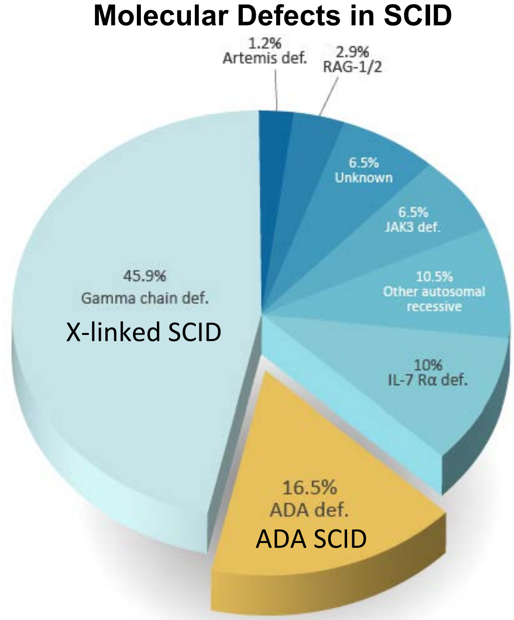
Case Study of Gene Therapy for Severe Combined Immunodeficiency (SCID)

Severe Combined Immunodeficiency Diseases (SCID)



A group of rare, sometimes fatal, congenital disorders characterized by little or no immune response.

Relative Frequency of the Different Molecular Defects in SCID



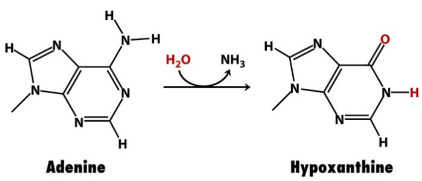
Types of SCIDs

- Adenosine deaminase deficiency
- X-linked severe combined immunodeficiency
- Purine nucleoside phosphorylase deficiency
- Reticular dysgenesis
- Omenn syndrome
- Bare lymphocyte syndrome
- JAK3
- Artemis/DCLRE1C

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

- 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-SCID patients can be treated with PEG-ADA, a stabilized form of the enzyme

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



Degradation of Purine

Treatments for ADA-SCID

	Bone Marrow Transplant (non-HLA identical sibling donor)	Gene Therapy	(PEG-ADA) Adagen
Type of therapy[5]	Replacement of host immune system by donor hematopoietic stem cells	Genetic modification of patient stem cells, autologous transplant	Enzyme replacement therapy
Goal[5,6]	Cure	Cure	Management
Patient selection[2,4,6]	Pts must be stabilized prior to transplant; higher success rate in younger pts	Pts must be stabilized prior to treatment	Pts can be treated within days of diagnosis

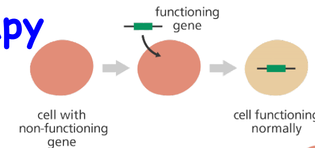
Questions to Consider
Before Initiating Gene Therapy

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?

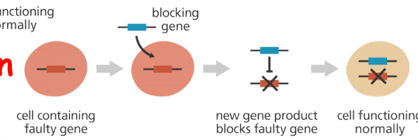
Types of Gene Therapy

- **Germline gene therapy**

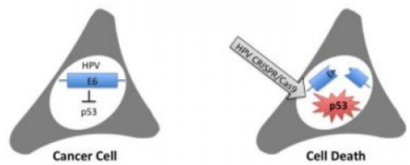
- **Somatic gene therapy**
  - Gene augmentation



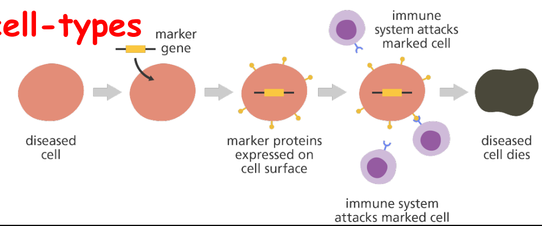
  - Targeted silencing of gene expression



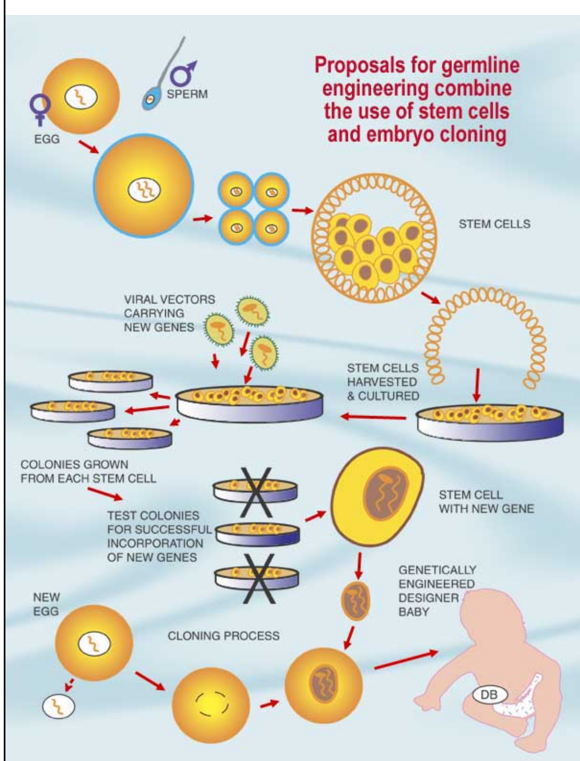
  - Gene alteration
  - Gene replacement



  - Targeted killing of specific cell-types



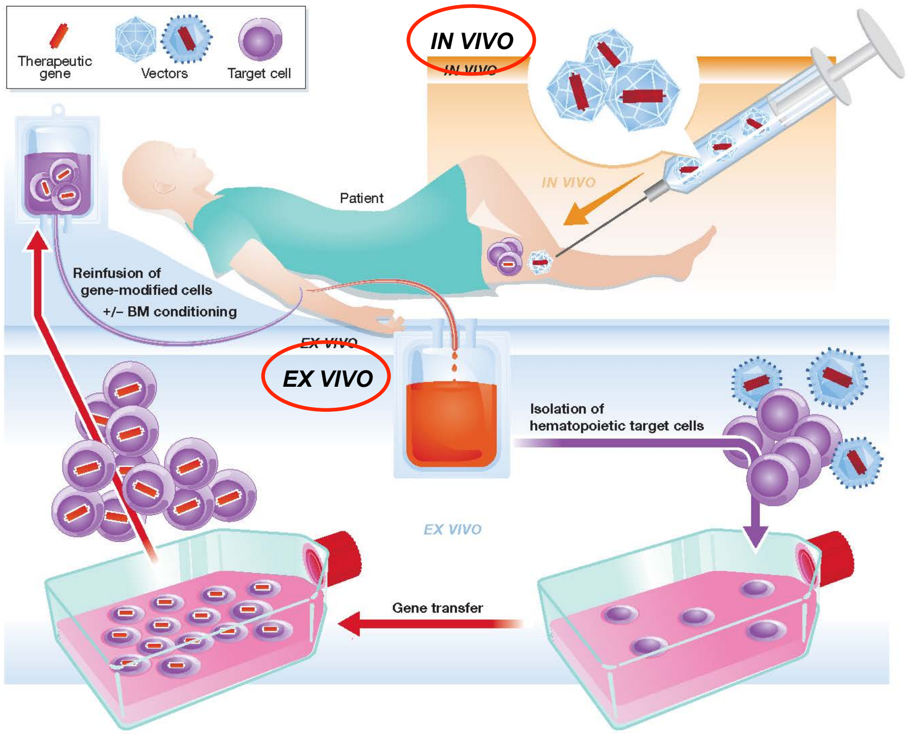
Germline Gene Therapy

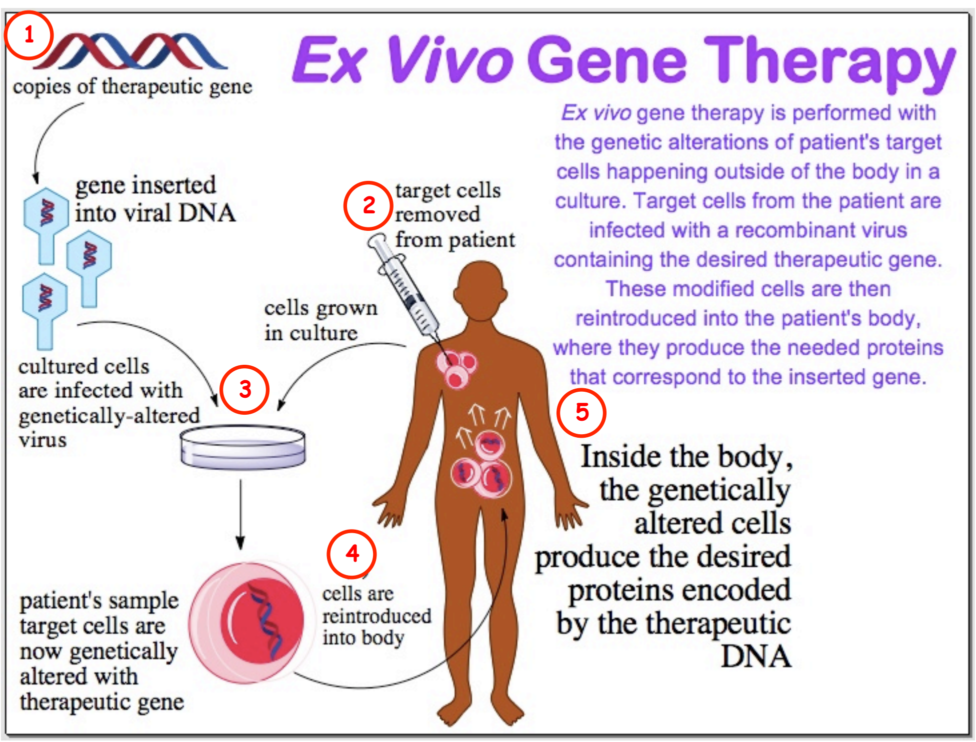


- Germline gene therapy is when DNA is transferred into the cells that produce reproductive cells, eggs or sperm, in the body. This type of therapy allows for the correction of disease-causing gene variants that are certain to be passed down from generation to generation
- It is NOT ILLEGAL to conduct 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.
- FDA cannot review applications for clinical trials that involve human embryos with heritable genetic modifications

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

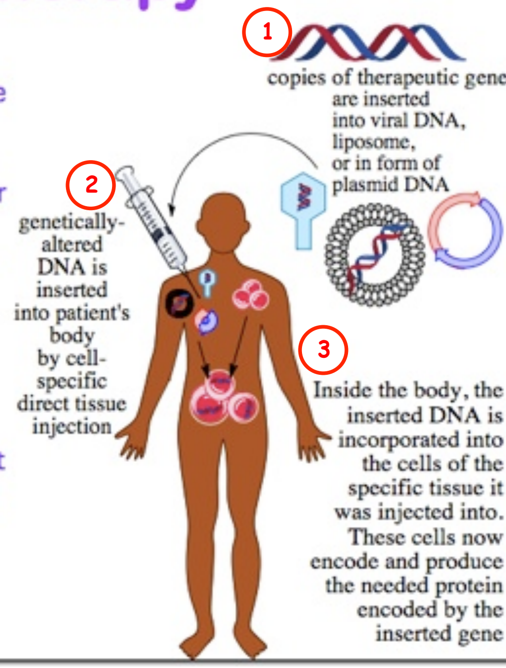
Somatic cell - any cell of a living organism other than the reproductive cells





In Vivo Gene Therapy

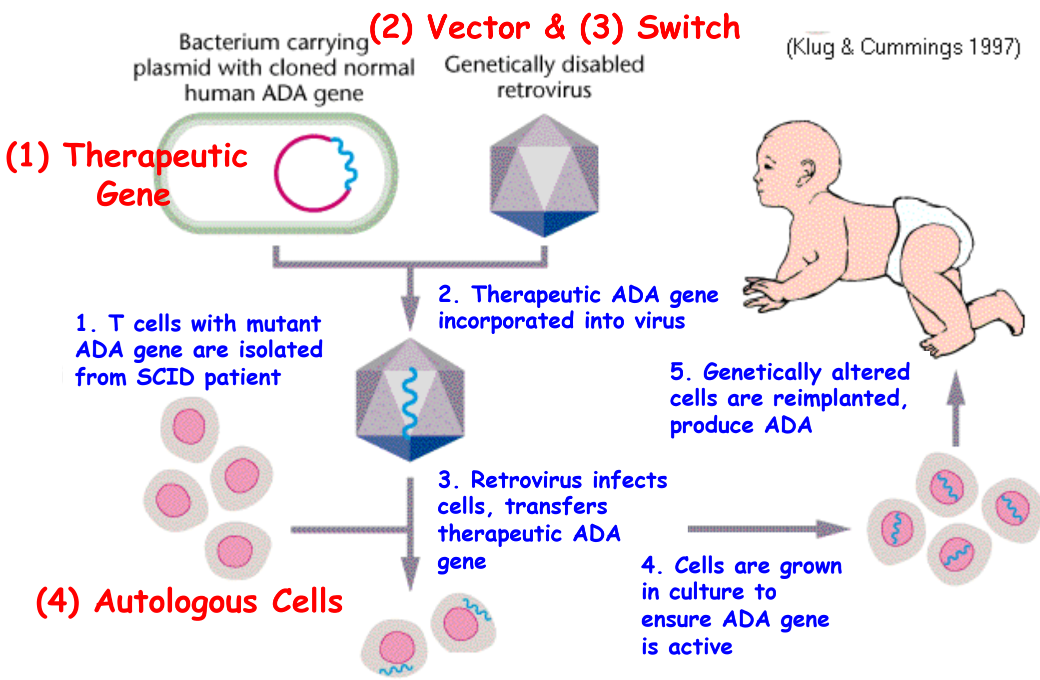
In vivo gene therapy involves introduction of therapeutic DNA directly into the patient's body. The DNA is introduced by cell-specific direct injection into tissue in need. DNA in the form of a plasmid vector is introduced by a dermal vaccination. Modified liposomes are not currently used for gene therapy,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



Toolkit for Ex Vivo Somatic Cell Gene Therapy Procedures

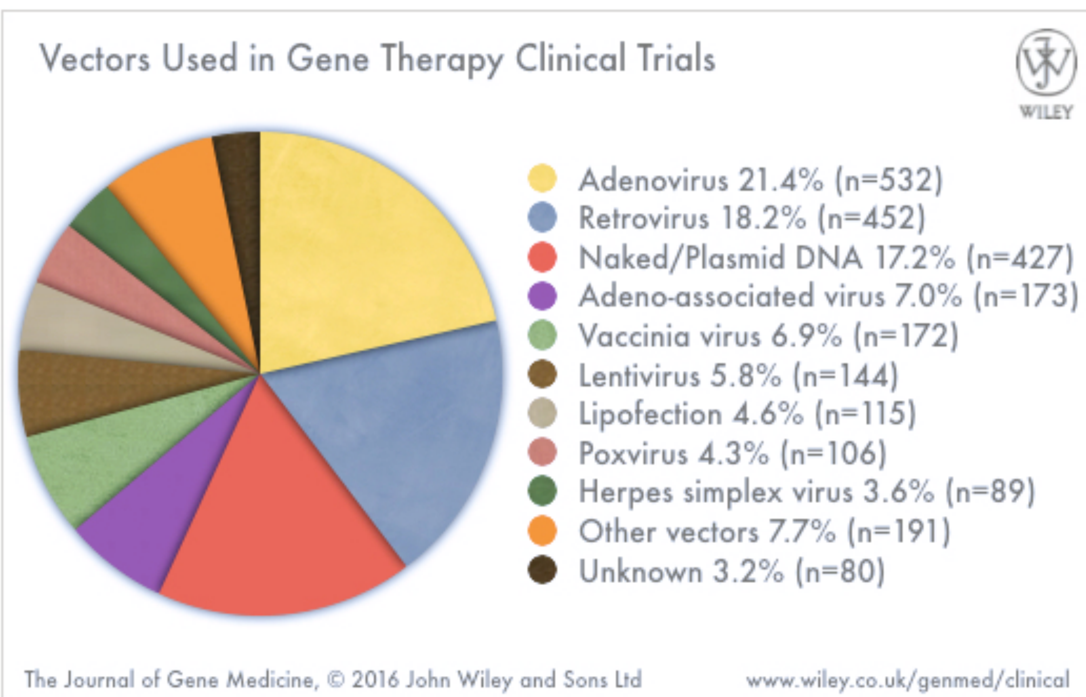
1. Cloned copy of the therapeutic gene
2. Appropriate switch - often a strong switch to drive high level expression of the gene
3. Vector to transfer the gene into the cells
4. Autologous cells (obtained from the same individual) or non-autologous cells

Ex Vivo Gene Therapy for ADA-Severe Combined Immunodeficiency (SCID)



ADA-SCID Clinical Trial Started in 1990

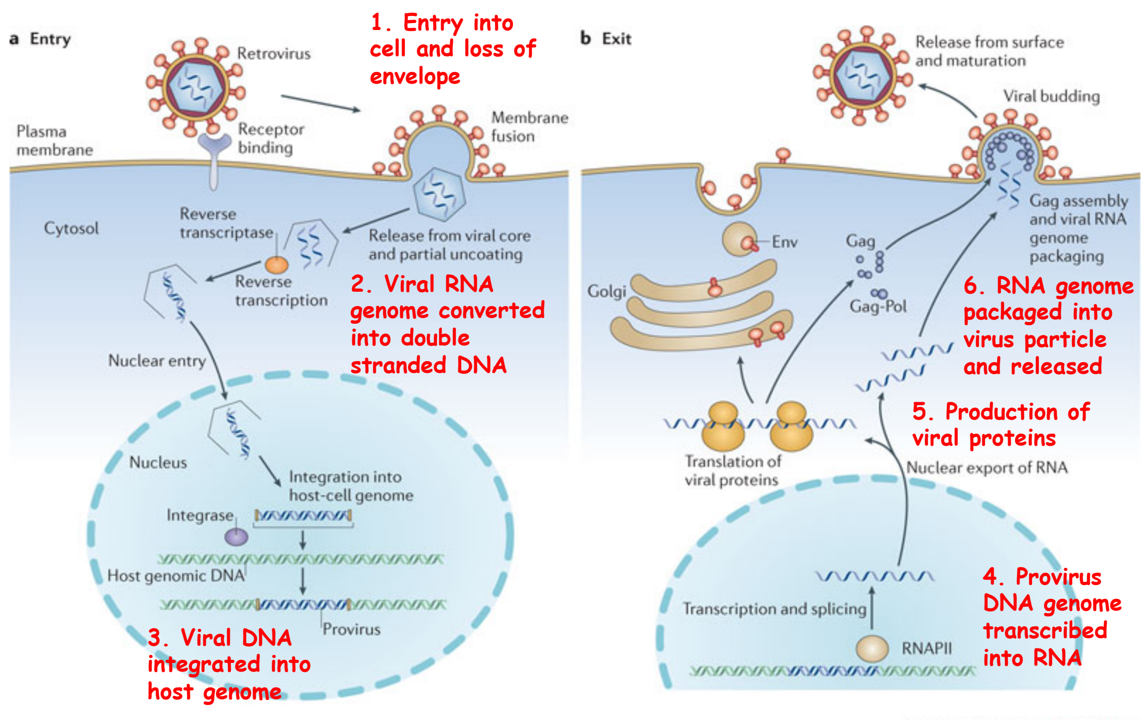
How are Therapeutic Genes Targeted and Delivered to Cells of Interest – with Vectors



Viral vector	Type	Advantages	Disadvantages
Retrovirus	Integrates with host chromatin	Effective over long periods Efficient transfection *ex vivo* Low immune response in host Transfects proliferating hosts	Small, max 8kb insert size Inefficient transfection *in vivo* Relies on target cell mitosis Safety concerns
Lentivirus	Integrates with host chromatin	Transfects proliferating and non -proliferating hosts and haemo stem cells New generations are self-inactivating for safety	Need active transport into cell Small, max 8kb insert size Technologically challenging Safety concerns, immunodeficiency origins
Adeno-Associated Virus	Either	Very good length of expression especially *in vivo* Efficient transfection *in vivo* Low immune response in host Transfects both types of hosts	Safety problems owing to potential insertional mutagenesis Small, max 4.5kb insert size High immuno response Technologically challenging
Adenovirus	Extra chromosomal DNA	Highly efficient transfection *in vivo* and *ex vivo* Transfects proliferating and non-proliferating hosts	Repeat treatments ineffective due to strong immune response Small, max 7.5kb insert size Technologically challenging Short expression duration
Herpes simplex virus	Extra chromosomal DNA	Very good length of expression especially *in vivo* Safe for use in immunocompromised patients Large insert size up to 30 kb Effective on many cell types	Difficult to produce in large quantities

Vectors Used to Deliver Genes to Cells in Gene Therapy

Retrovirus Life Cycle

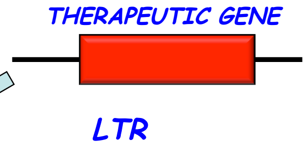


Retroviruses Replicate Using Reverse Transcriptase

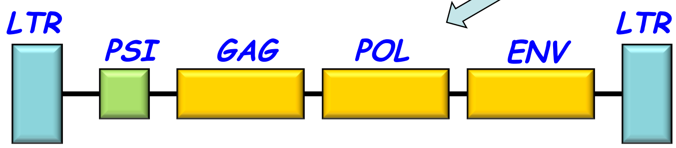
David Baltimore & Howard Temin-Nobel Prize 1975

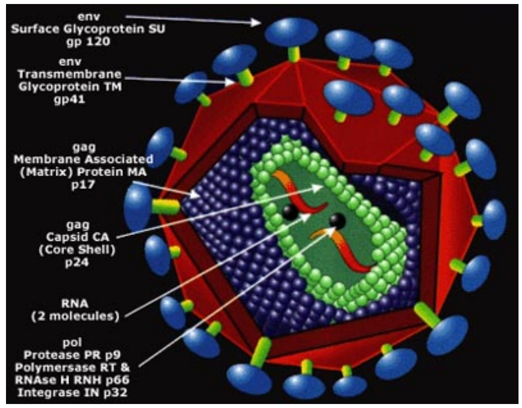
Modification of the Central Dogma of Molecular Biology - RNA to DNA to RNA to Protein

Retroviral Vector

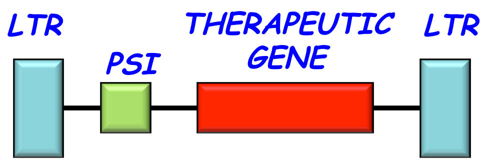


Retrovirus





Retroviral Gene Therapy Vector



Did the Gene Therapy Strategy Work?

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

Cindy Kisik & Ashanthi DeSilva 1992

Ashanthi DeSilva, Michael Blasé, & Cindy Kisik 2013

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

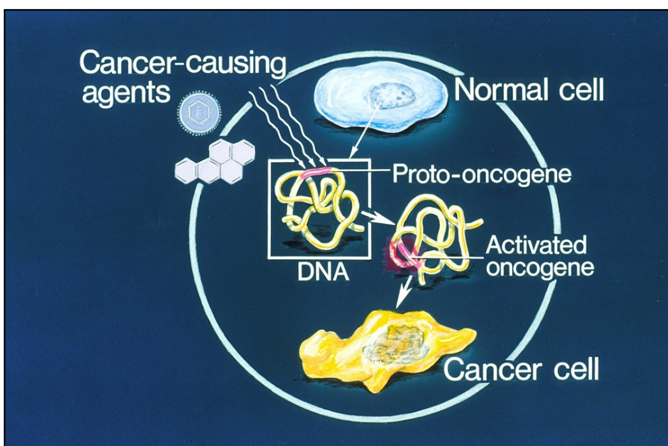
TRIALS ARE HALTED ON A GENE THERAPY

By SHERYL GAY STOLBERG
Published: October 4, 2002

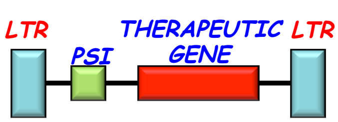
WASHINGTON, Oct. 3— Officials in the United States and France said today that they had suspended four gene therapy experiments because the treatment, which cured a 3-year-old boy of a fatal immune deficiency, may have given him an illness similar to leukemia.

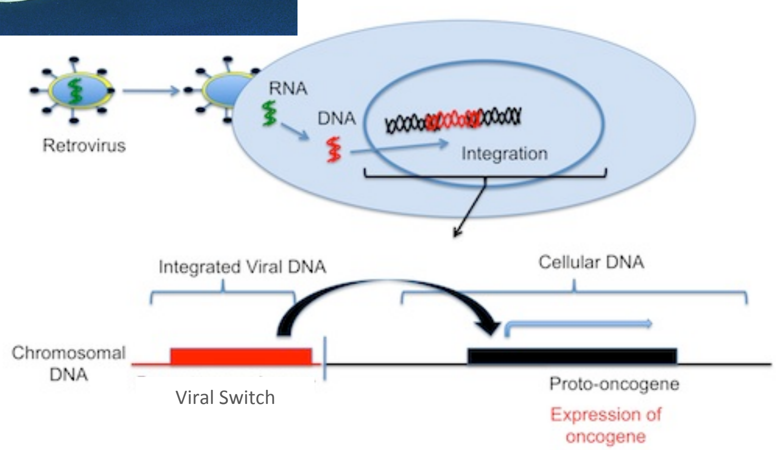
- 3 of 17 patients in clinical trial for X-SCID gene therapy developed clonal lymphoproliferative disorder – a leukemia
- The leukemia was caused by insertion of retrovirus near proto-oncogenes and activation of these proto-oncogenes by retroviral switches

Retroviral Switches can Activate Proto-oncogenes and Induce Cancers



A Typical Retrovirus Gene Therapy Vector





REPORT AND RECOMMENDATIONS OF THE PANEL TO ASSESS THE NIH INVESTMENT IN RESEARCH ON GENE THERAPY

Stuart H. Orkin, M.D. Arno G. Motulsky, M.D.
Co-chairs
December 7, 1995

MAJOR RECOMMENDATIONS

- In order to confront the major outstanding obstacles to successful somatic gene therapy, greater focus on basic aspects of gene transfer, and gene expression within the context of gene transfer approaches, is required. Such efforts need to be applied to improving vectors for gene delivery, enhancing and maintaining high level expression of genes transferred to somatic cells, achieving tissue-specific and regulated expression of transferred genes, and directing gene transfer to specific cell types.
- To address important biological questions and provide a basis for the discovery of alternative treatment modalities, the Panel recommends increased emphasis on research dealing with the mechanisms of disease pathogenesis, further development of animal models of disease, enhanced use of preclinical gene therapy approaches in these models, and greater study of stem cell biology in diverse organ systems
- Strict adherence to high standards for excellence in clinical protocols must be demanded of investigators. Gene therapy protocols need to meet the same high standards required for all forms of translational (or clinical) research, whatever the enthusiasm for this (or any other) treatment approach.

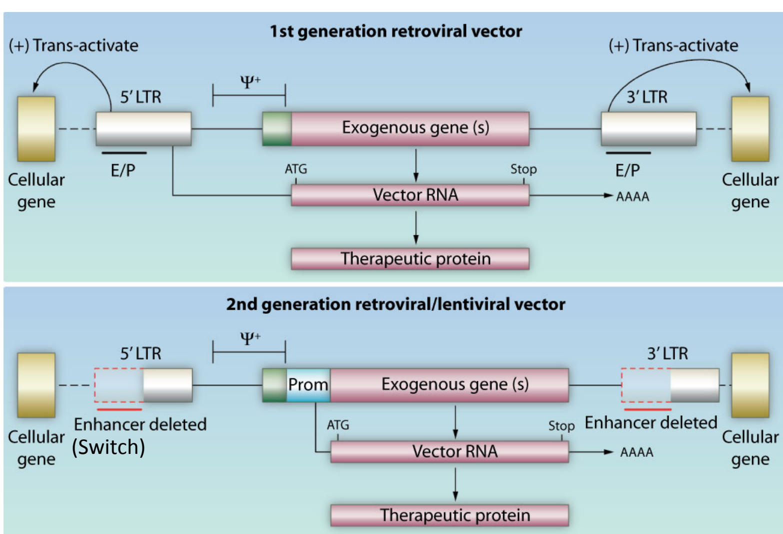
Some Early Problems with Human Gene Therapy

- **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)**
- **Incomplete understanding of disease biology**
- **Human error - failure to adhere to strict NIH and IRB procedures (experimental therapies)**

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

ScienceDaily

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HEALTH PHYSICAL/TECH ENVIRONMENT SOCIETY/EDUCATION QUIRKY

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.

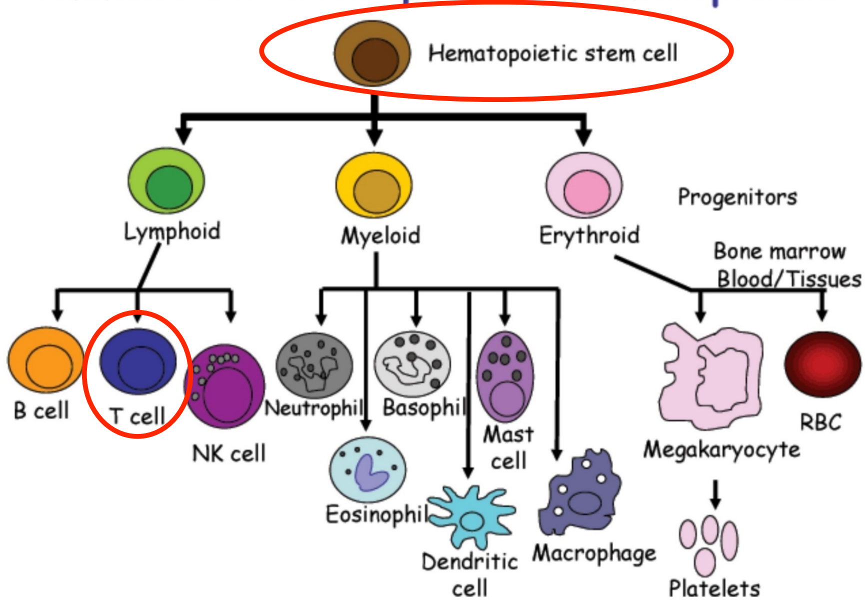
[a] Associated with high frequency of SAEs (5 out of 19).

[b] Associated with very high frequency of SAEs (seven out of nine for WAS, and four out of four for CGD).

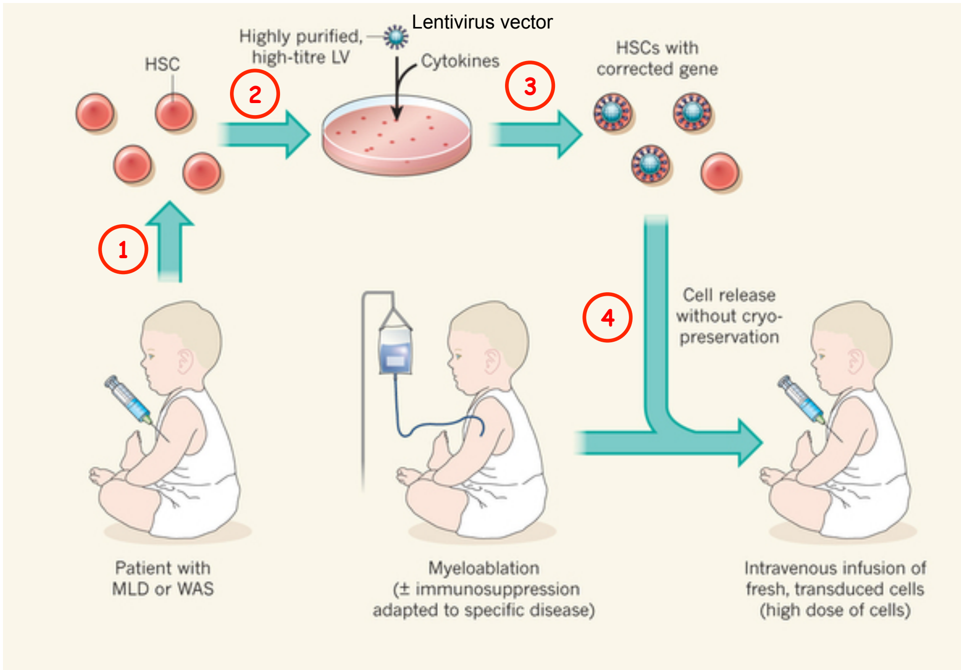
[c] CD34 and T cell strategy are both envisaged.

Using Stem Cells in *Ex Vivo* Gene Therapy

Immune cell development: Hematopoiesis

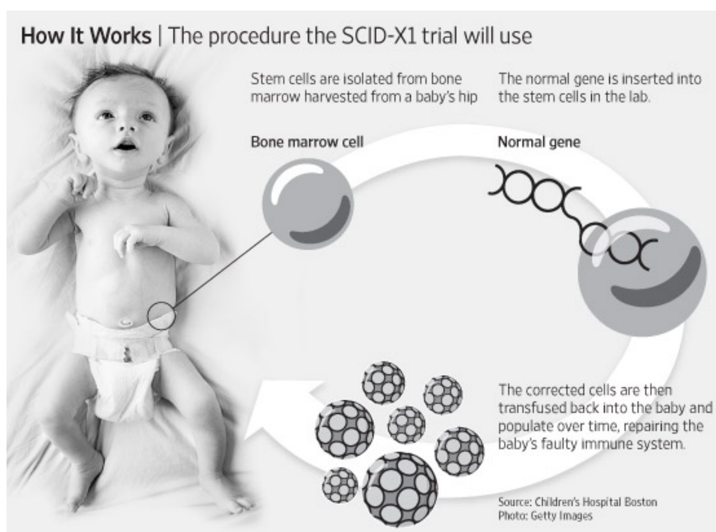


General Strategy for Use of Hematopoietic Stem Cells in Gene Therapy



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

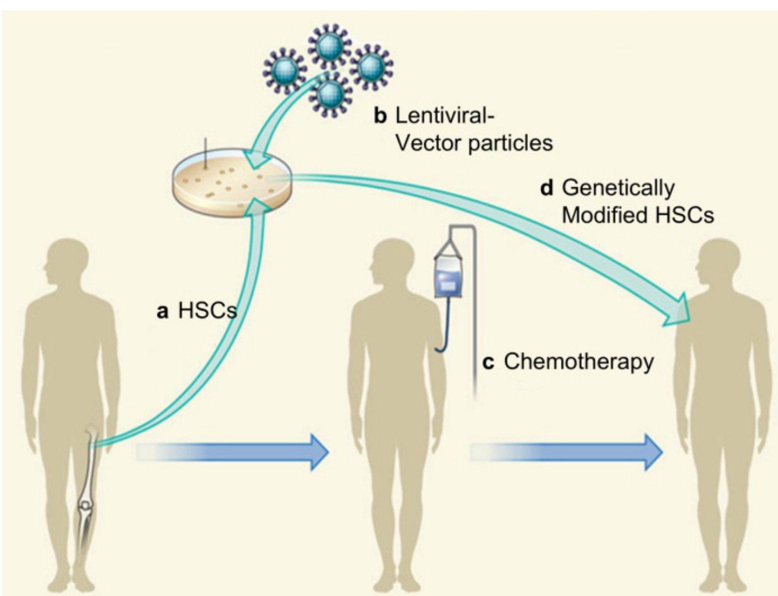
- ADA-SCID – 4 of 6 children experienced immune reconstitution
- SCID-X1 – 9 of 10 children experienced normal T-cell number
- But - 5 of 20 SCID-X1 subjects experienced leukemia-like T lymphoproliferation in another study

Strimvelis®

EMA APPROVED

- ADA-SCID gene therapy product named Strimvelis from GlaxoSmithKline
- Approved for use in Europe in May 2016, first used March 2017
- One time treatment costs $714,000, 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



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

Regulation of Gene Therapy

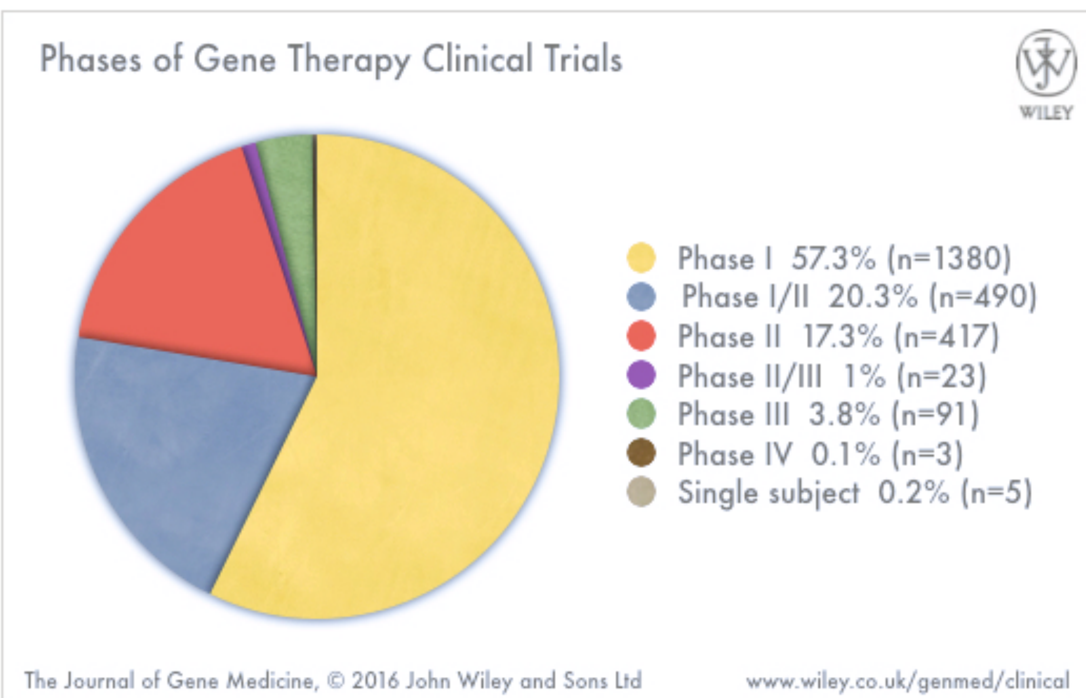
US Regulatory Authority for Gene Therapy

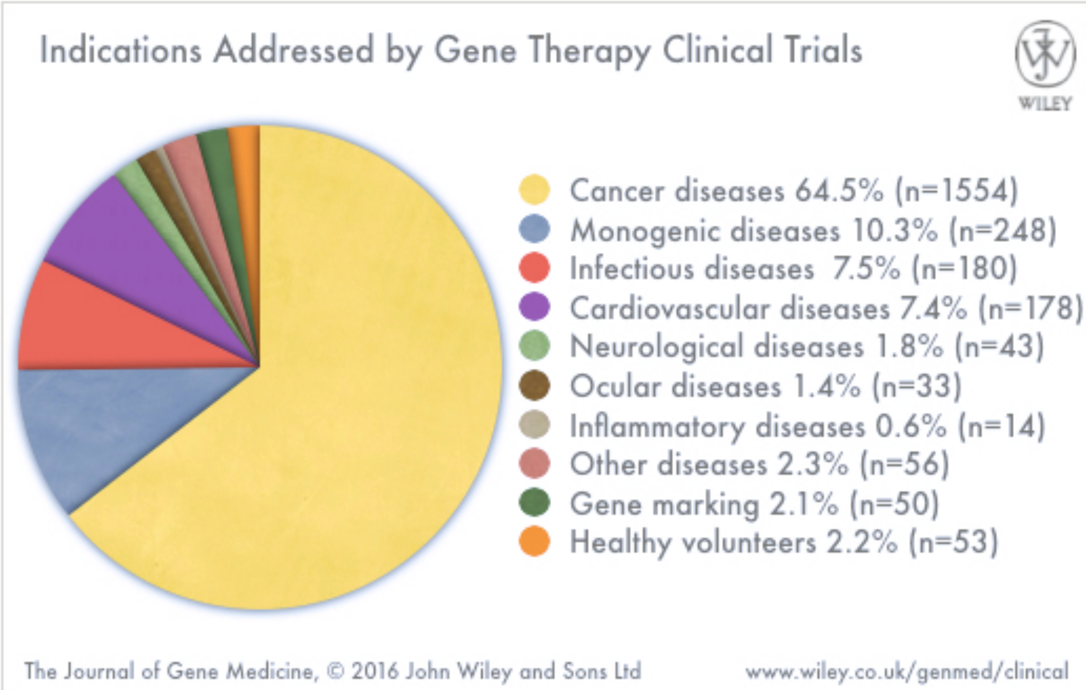
- Department of Health and Human Services (DHHS) has been charged with oversight of clinical trials
  - Office for Human Research Protections
    - All research involving human subjects undergo Institutional Review Board review
  - U.S. Food and Drug Administration
    - Center for Biologics Evaluation 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
    - FDA cannot review applications for clinical trials that involve human embryos with heritable genetic modifications
- 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

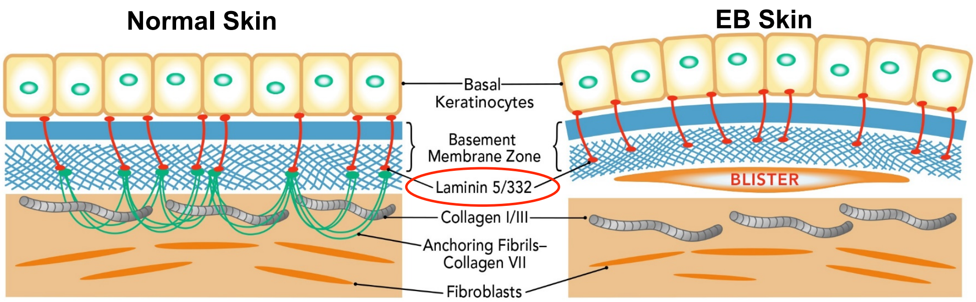




Ex Vivo Gene Therapy to Make Transgenic Skin

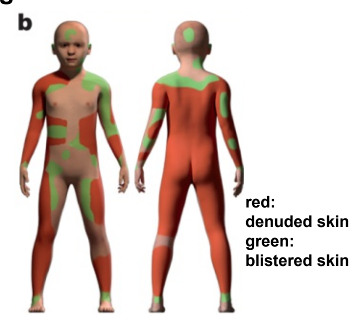
Epidermolysis Bullosa – Butterfly Disease

Epidermolysis bullosa is a rare group of inherited conditions that causes a person to develop blisters in the skin and mucosal membranes when they come into contact with heat, friction, rubbing, scratching, or minor injury.



Junctional EB is caused by mutations in genes for laminin 5/332

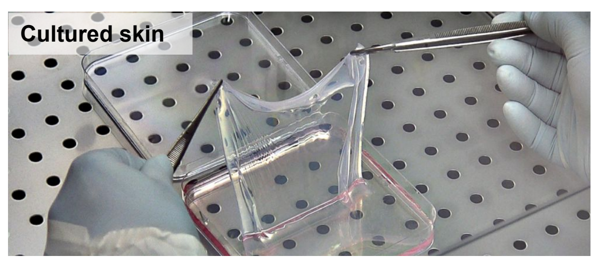
Patient with mutation in *LAMB3* gene with blistered & denuded skin over 80% of his body

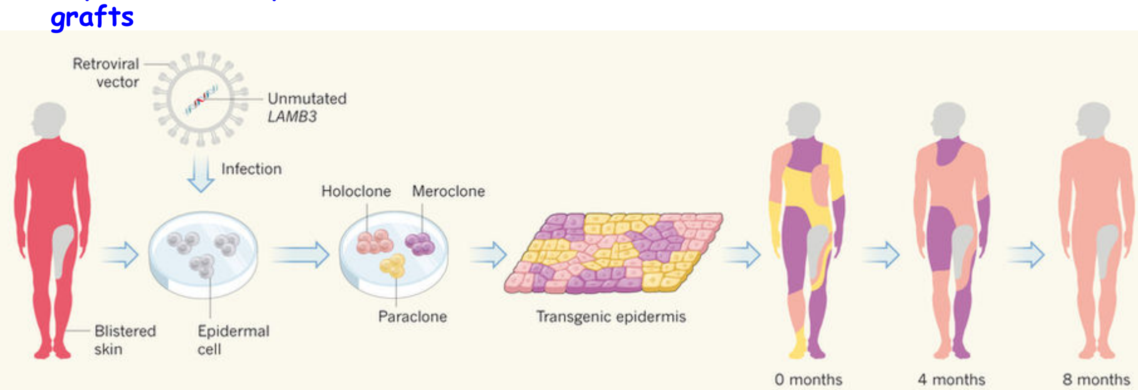


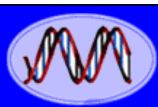
Ex Vivo Gene Therapy for Junctional Epidermolysis Bullosa

Protocol

- Removed non-diseased skin from patient
- Transfered normal *LAMB3* gene into epidermal cells
- Grew transgenic epidermis - holoclone (stem cell), meroclone (transition cell), & paraclone (differentiated) - in culture
- Replace 80% of patient's skin with grafts



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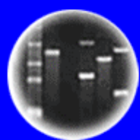
Genetic Code of Life


Entire Genetic Code
of a Bacteria


DNA Fingerprinting


Cloning: Ethical Issues
and Future Consequences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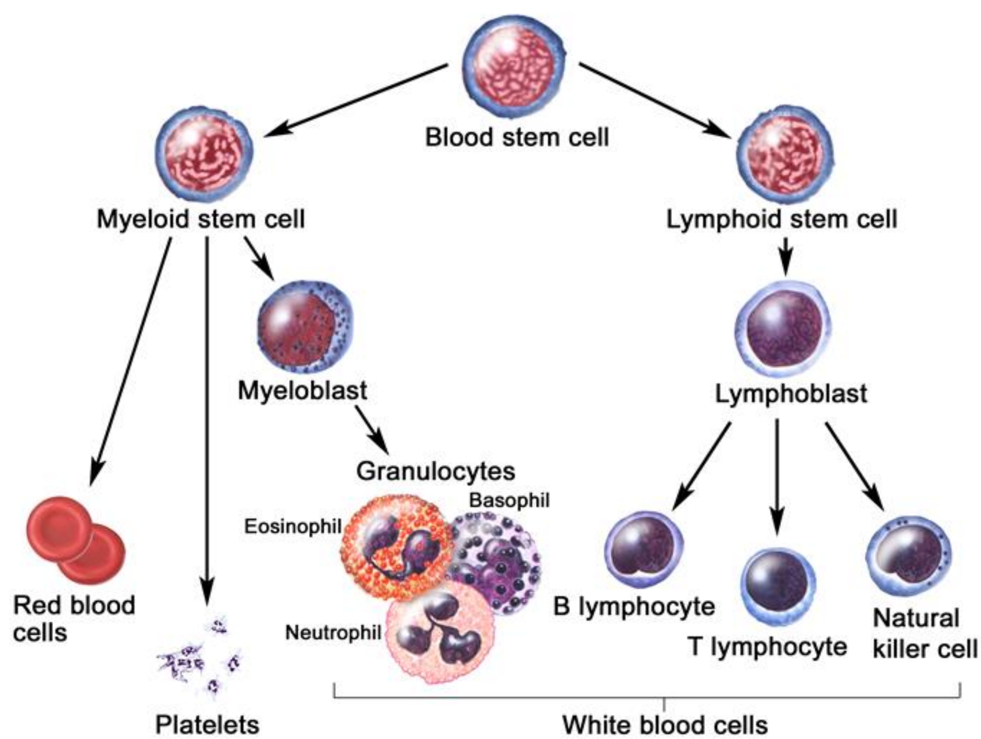
Ex Vivo Gene Therapy to Control Cancers

Normal Blood Cell Development



The New York Times

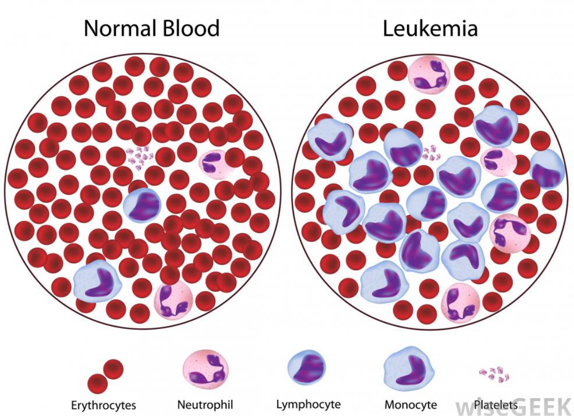
December 9, 2012

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

By DENISE GRADY

2013

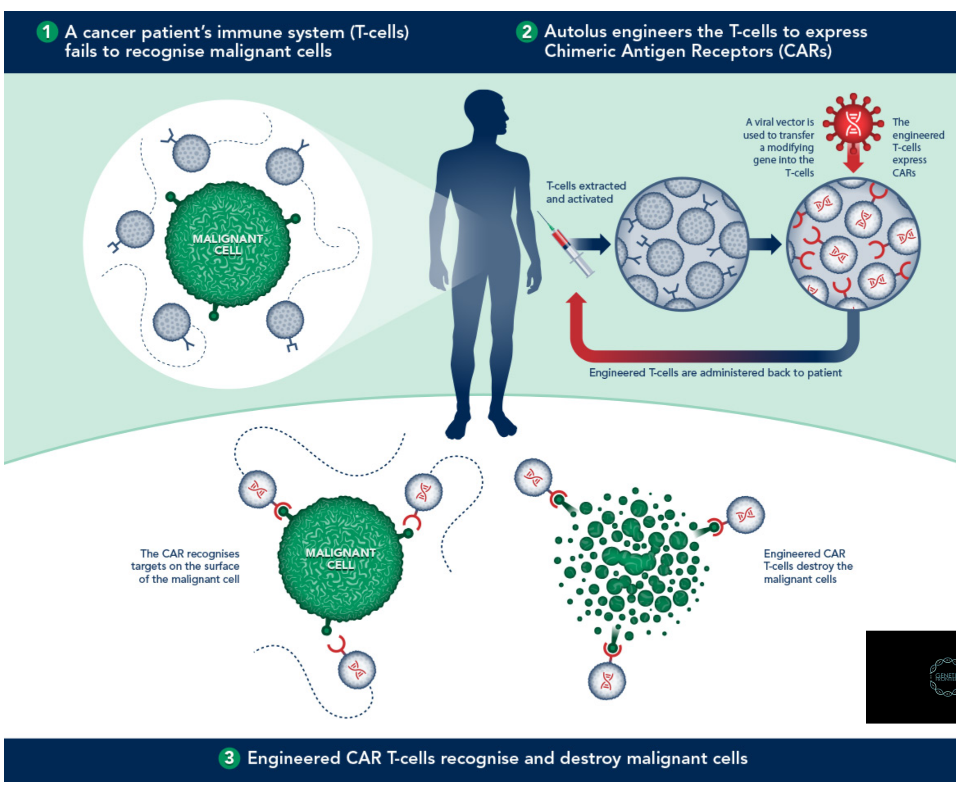
Leukemia is cancer of the blood, that results in an increase in immature white blood cells. Chronic lymphoid leukemia affects B cell lymphocytes



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

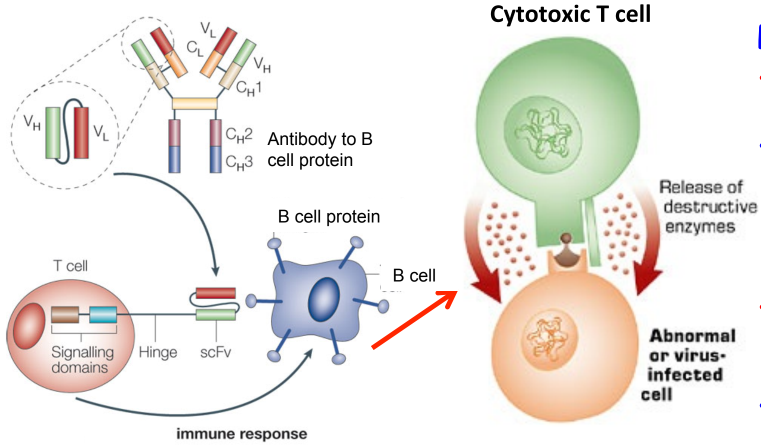
Emily Whitehead, alive at age 7 because of a novel gene therapy strategy

Chimeric Antigen Receptor (CAR) Strategy



Ex-vivo Gene Therapy for Lymphocytic Leukemia

Science Translational Medicine AAAS

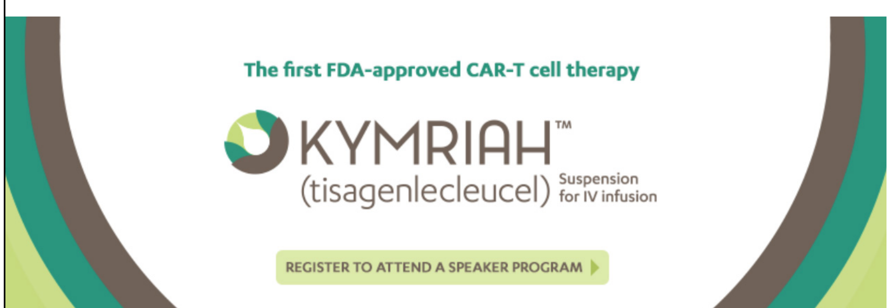


Protocol

- 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

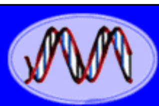
Two CAR-T Treatments were the First Gene Therapies to be FDA Approved in 2017



- Treatment for B-cell acute lymphoblastic leukemia
- Approved August 30, 2017
- $475,000 per treatment course

- Treatment for non-Hodgkin lymphoma
- Approved October 18, 2017
- $373,000 per treatment course

YESCARTA™ is a treatment for your non-Hodgkin lymphoma. It is used when you have failed at least two other kinds of treatment. YESCARTA™ is different than other cancer medicines because it is made from your own white blood cells, which have been modified to recognize and attack your lymphoma cells.



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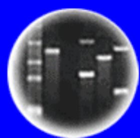

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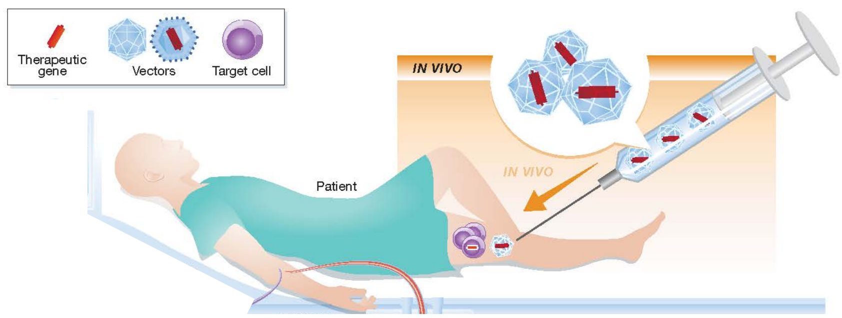
In Vivo
Gene Therapy

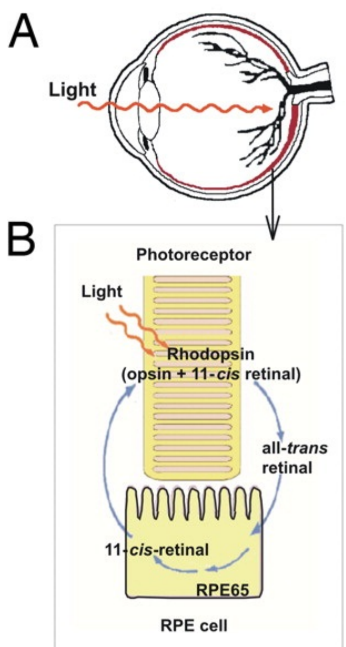


Toolkit for In Vivo Somatic Cell Gene Therapy Procedures

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 - Leber Congenital Amaurosis (LCA)

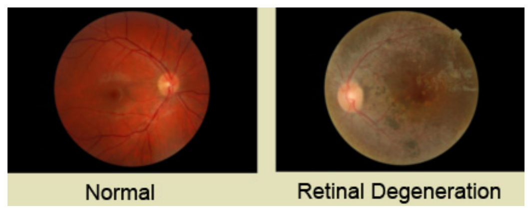
How We See



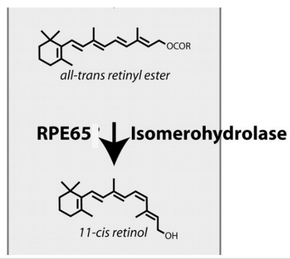
Cideciyan et al. PNAS 2008;105:15112

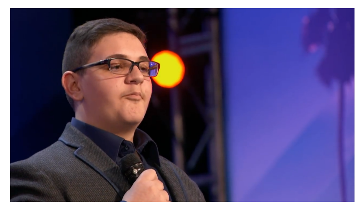
- Degenerative disease of the retina
- The most common cause of congenital blindness in children

Normal retina | LCA retina



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



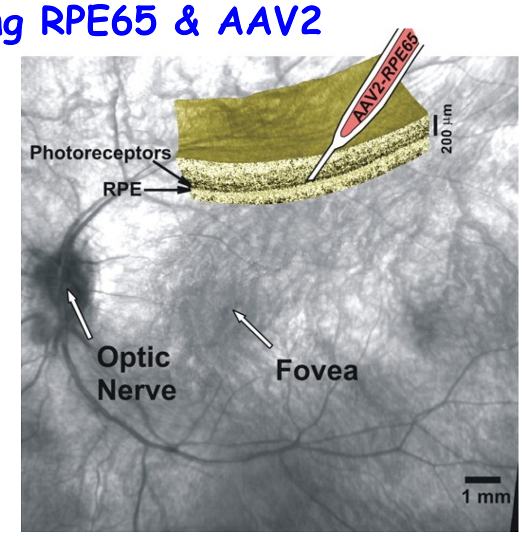


Christian Guardino

LCA Gene Therapy Using RPE65 & AAV2

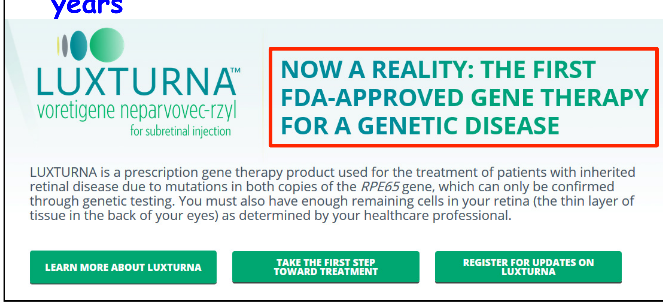
Protocol

- Subretinal injection of adeno-associated viruses (AAV2) with RPE65 gene. AAVs…
  - do not generally provoke antibody formation
  - infects nondividing cells of many different tissues
  - has little or no integration of viral DNA into the host genome



Results

- Patients showed statistically significant improvement in vision in Phase 3 clinical trials, with 65% showing maximum possible improvement
- Improvements maintained up to three years

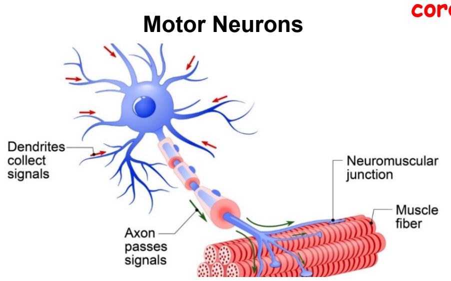


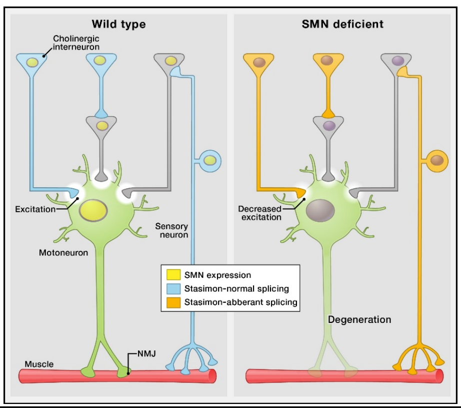
- Approved December 19, 2017
- $425,000 per eye
- Money-back guarantee

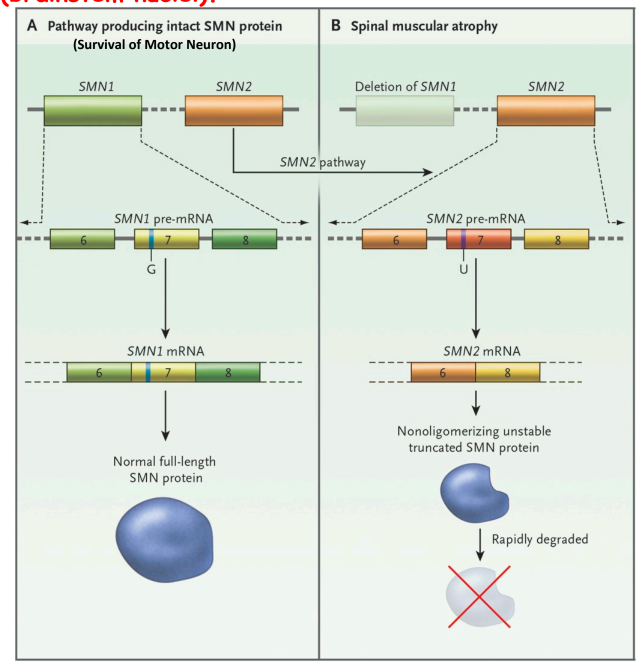
"I was able to see things for the first time — like the moon. I was able to see stars for the first time – fireworks — all these amazing things that I've never been able to see before."

Spinal Muscular Atrophy

Spinal Muscular Atrophy is an autosomal recessive neurodegenerative disease, and the most common cause of mortality in infants linked to a genetic mutation. The disease is characterized by progressive muscle weakness caused by a loss of specialized nerve cells (motor neurons) in the spinal cord and the part of the brain that is connected to the spinal cord (brainstem nuclei).







In-vivo Gene Therapy for Spinal Muscular Atrophy Type 1

Protocol for Phase 1 Clinical Trial

- Transferred the SMN gene into the AAV9 vector
  - AAV9 when infused into a vein can move across the blood-brain barrier to the central nervous system
- Patients were given a single of intravenous AAV9-SMN treatment – 3 at a low dose and 12 at a high dose

Results

- All 15 children treated were alive at 20 months or older and did not require ventilation
  - Other studies show that only 8% of untreated children survive to 20 months without ventilation
- Of 12 patients given the high dose, 11 sat unassisted, 9 rolled over, 11 fed orally and could speak, and 2 walked independently

Evelyn Villarreal

However….

Human Gene Therapy

Severe Toxicity in Nonhuman Primates and Piglets Following High-Dose Intravenous Administration of an Adeno-Associated Virus Vector Expressing Human SMN

To cite this article:
Hinderer Christian, Katz Nathan, Buza Elizabeth L., Dyer Cecilia, Goode Tamara, Bell Peter, Richman Laura K., and Wilson James M.. Human Gene Therapy. February 2018, ahead of print. https://doi.org/10.1089/hum.2018.015

Online Ahead of Print: February 12, 2018
Online Ahead of Editing: January 29, 2018

Treated non-human primates and piglets with high doses of AAV9-SMN

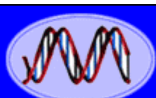
- Non-human primates experienced severe liver toxicity
- Piglets exhibited loss of control of bodily movements

IN DEPTH | BIOMEDICINE

Gene therapy field hit by fresh safety concern

Jocelyn Kaiser
+ See all authors and affiliations

Science 09 Feb 2018:
Vol. 359, Issue 6376, pp. 621
DOI: 10.1126/science.359.6376.621



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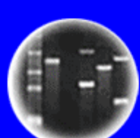


DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

Current Status of Gene Therapy

Approved Gene Therapy Products Worldwide

赛百诺 SIBIONO
P53 tumor suppressor deficiency
Marketed in China 2004

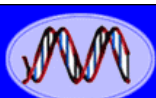
~~uniQure~~
~~Lipoprotein lipase deficiency~~
~~Marketed in Europe 2012~~

Strimvelis®
ADA-SCID
Marketed in Europe 2016
EMA APPROVED

LCA Blindness
FDA Approved 2017
LUXTURNA™
voretigene neparvovec-rzyl
for subretinal injection

2017
Introducing the first
FDA-approved CAR-T cell therapy:
CTL019 is now
KYMRIAH™
(tisagenlecleucel) Suspension for IV infusion

NOW APPROVED
CAR-T Therapy
FDA Approved 2017
YESCARTA™
(axicabtagene ciloleucel) Suspension for IV infusion



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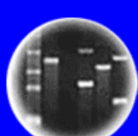

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Issues Concerning Gene Therapy

Gene Therapy for Human Genetic Disease?

3 March 1972, Volume 175, Number 4025 SCIENCE

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

Theodore Friedmann and Richard Roblin

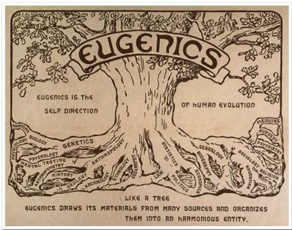
We propose the following ethico-scientific criteria which any prospective techniques for gene therapy in human patients should satisfy:

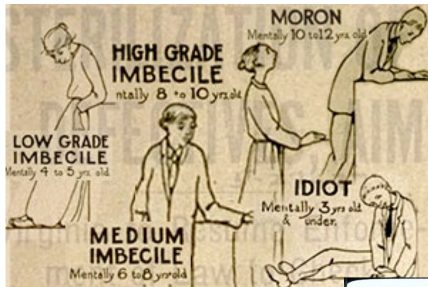
1. There should be adequate biochemical characterization of the prospective patient's genetic disorder.
2. There should be prior experience with untreated cases of what appears to be the same genetic defect
3. There must be an adequate characterization of the quality of the exogenous DNA vector.
4. There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

- Germline Gene Therapy
- Eugenics & the "Slippery Slope" Towards Enhancement
- Consent
- Availability To Everyone $$$

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

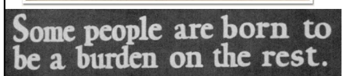


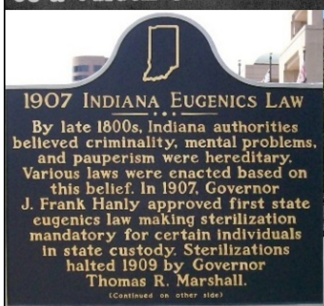


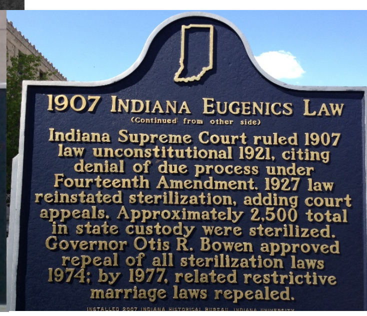

DECISION HELD STEP TOWARD A SUPER-RACE

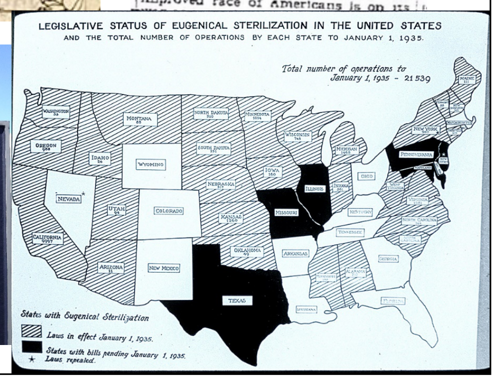
Health Official Praise Court in Upholding Sterilization Law

WASHINGTON, May 5.—(INS)—An improved race of Americans is on its









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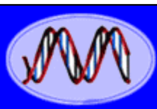

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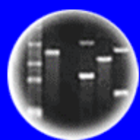

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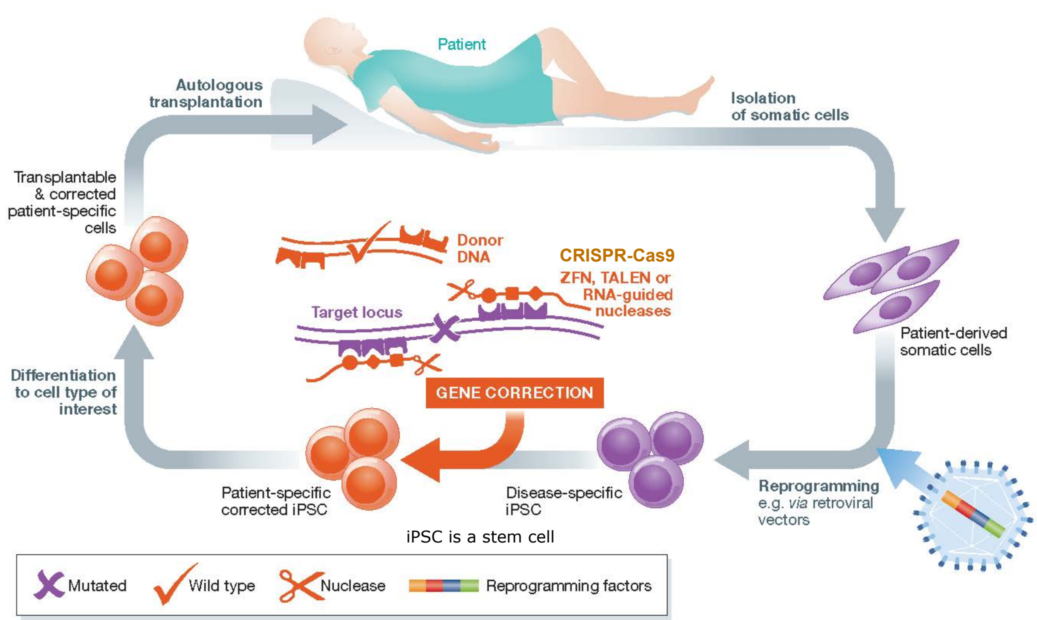
Gene Editing & Human Gene Therapy

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

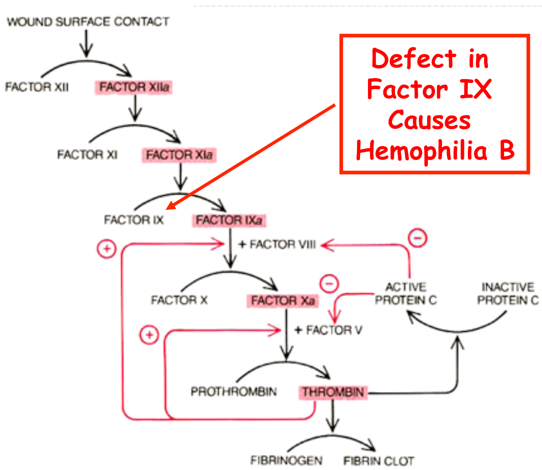
Xiao-Jie et al. 2015

The Scientist » News & Opinion » News Analysis

First In Vivo Human Genome Editing to Be Tested in New Clinical Trial

Sangamo Therapeutics will use zinc finger nucleases to introduce the gene for a missing clotting factor into the livers of men with hemophilia B.

By Abby Olena | May 18, 2017



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.

Gene Therapy

- Used zinc finger nucleases to correct a defect in the clotting Factor IX gene
- AAV vectors used to target the editing enzymes to the liver

SCIENCE & HEALTH
February 06, 2018 9:59 PM
Associated Press

Second Man Undergoes Gene Editing; Therapy Has No Safety Flags So Far

Gene Editing Trials in China

ClinicalTrials.gov

Row	Saved	Status	Study Title	Conditions	Interventions	Locations
1	☐	Not yet recruiting	A Safety and Efficacy Study of TALEN and **CRISPR**/Cas9 in the Treatment of HPV-related Cervical Intraepithelial NeoplasiaⅠ	• Human Papillomavirus-Related Malignant Neoplasm	• Biological: TALEN • Biological: **CRISPR**/Cas9	• The First Affiliated Hospital of Sun Yat-sen University Guangzhou, Guangdong, **China**
2	☐	Recruiting	Safety of Transplantation of **CRISPR** CCR5 Modified CD34+ Cells in HIV-infected Subjects With Hematological Malignances	• HIV-1-infection	• Genetic: CCR5 gene modification	• 307 Hospital of PLA (Affiliated Hospital of Academy to Military Medical Sciences) Beijing, Beijing, **China**
3	☐	Recruiting	Identification of Host Factors of Norovirus Infections in Mini-Gut Model	• Gastrointestinal Infection	• Procedure: Duodenal biopsy • Procedure: Saliva	• Endoscopy Centre, Prince of Wales Hospital Hong Kong, **China**
4	☐	Recruiting	A Study Evaluating UCART019 in Patients With Relapsed or Refractory CD19+ Leukemia and Lymphoma	• B Cell Leukemia • B Cell Lymphoma	• Biological: UCART019	• Biotherapeutic Department and Hematology Department of Chinese PLA General Hospital Beijing, Beijing, **China**
5	☐	Recruiting	A Feasibility and Safety Study of Universal Dual Specificity CD19 and CD20 or CD22 CAR-T Cell Immunotherapy for Relapsed or Refractory Leukemia and Lymphoma	• B Cell Leukemia • B Cell Lymphoma	• Biological: Universal Dual Specificity CD19 and CD20 or CD22 CAR-T Cells	• Biotherapeutic Department and Hematology Department of Chinese PLA General Hospital Beijing, Beijing, **China**
6	☐	Recruiting	PD-1 Knockout Engineered T Cells for Advanced Esophageal Cancer	• Esophageal Cancer	• Other: PD-1 Knockout T Cells	• Hangzhou Cancer Hospital Hangzhou, Zhejiang, **China**
7	☐	Not yet recruiting	PD-1 Knockout Engineered T Cells for Muscle-invasive Bladder Cancer	• Invasive Bladder Cancer Stage IV	• Biological: PD-1 Knockout T Cells • Drug: Cyclophosphamide • Drug: IL-2	• Department of Urology Peking University First Hospital Beijing, Beijing, **China**
8	☐	Not yet recruiting	PD-1 Knockout Engineered T Cells for Castration Resistant Prostate Cancer	• Hormone Refractory Prostate Cancer	• Biological: PD-1 Knockout T Cells • Drug: Cyclophosphamide • Drug: IL-2	• Department of Urology Peking University First Hospital Beijing, Beijing, **China**
9	☐	Recruiting	PD-1 Knockout Engineered T Cells for Metastatic Non-small Cell Lung Cancer	• Metastatic Non-small Cell Lung Cancer	• Drug: Cyclophosphamide • Other: PD-1 Knockout T Cells	• West China Hospital, Sichuan University Chengdu, Sichuan, **China**
10	☐	Recruiting	PD-1 Knockout EBV-CTLs for Advanced Stage Epstein-Barr Virus (EBV) Associated Malignancies	• Stage IV Gastric Carcinoma • Stage IV Nasopharyngeal Carcinoma • T-Cell Lymphoma Stage IV • (and 2 more...)	• Drug: Fludarabine • Drug: Cyclophosphamide • Drug: Interleukin-2	• The Comprehensive Cancer Center of Nanjing Drum Tower Hospital Nanjing, Jiangsu, **China** • The Comprehensive Cancer Center of Nanjing Drum Tower Hospital Nanjing, Jiangsu, **China**

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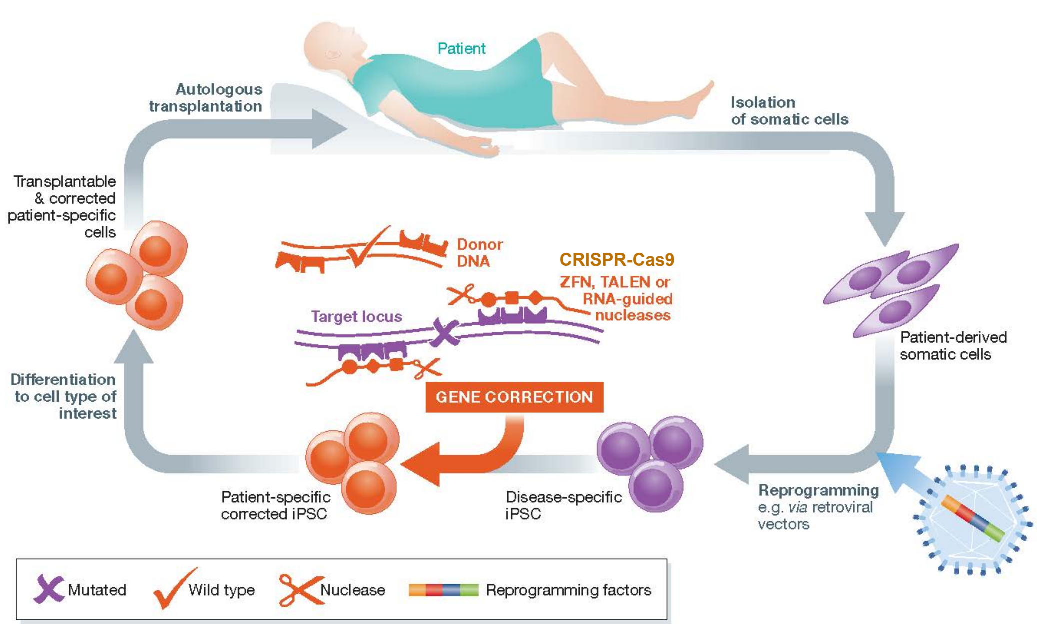
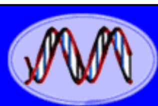
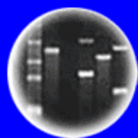




DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

The End!!

HC70A/SAS70A/PLSS059 Lectures on the History, Science, and Applications of Genomics & Genetic Engineering