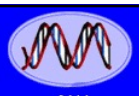


DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria

DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

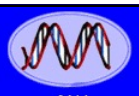
Plants of Tomorrow

HC70A & SAS70A
Winter 2023
Genetic Engineering in Medicine, Agriculture, and Law

Professors Bob Goldberg & John Harada

Lecture 9
Human Genetic Engineering and Gene Therapy

1



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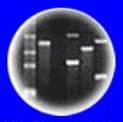

DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

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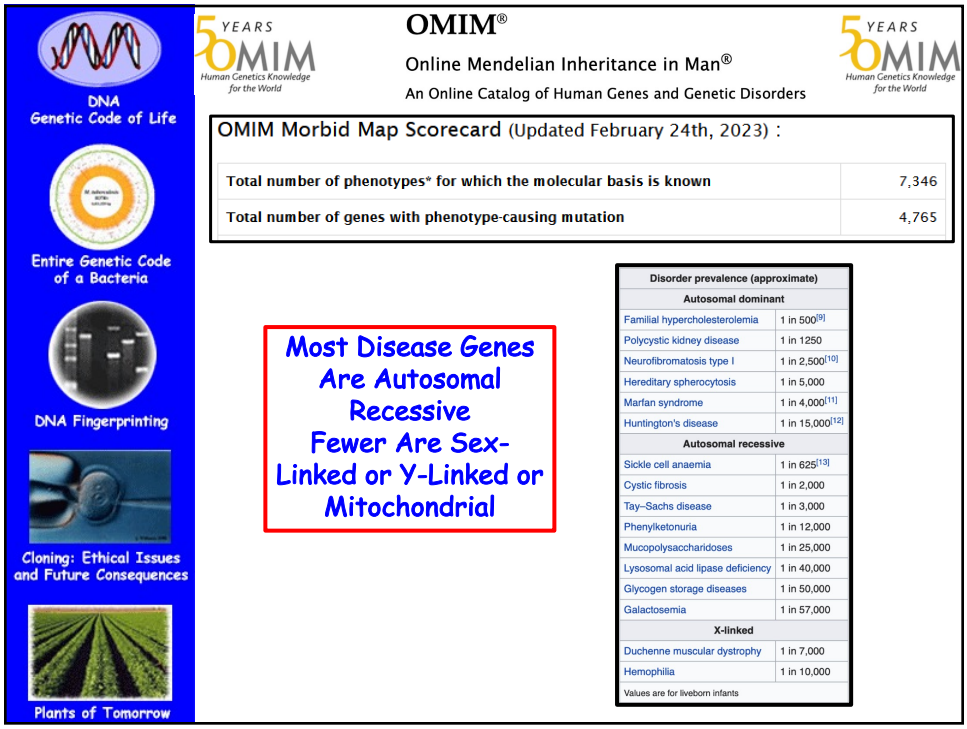
THEMES

1. What is the Spectrum of Human Disease Genes?
2. How are Human Disease Genes Inherited?
3. What are Treatments For Disease Genes Discussed in HC70A?
4. What are the Different Forms of Gene Therapy and What Types of Genes Can They Treat?
5. What are the Different Types of Gene Therapy?
6. Germline Gene Therapy
7. Somatic Cell Gene Therapy
    a. Ex Vivo
    b. In Vivo
8. What Vectors are Used For Gene Therapy?
9. Using Gene Therapy to Treat SCID-ADA
    a. What is SCID-ADA?
    b. Retrovirus Genome & Life Cycle
    c. Gene Therapy For SCID-ADA
    d. Problems & Solutions
10. Ex Vivo Gene Therapy for Cancer – CAR-T
11. In Vivo Gene Therapy
12. ASO Gene Silencing For Dominant Genetic Disorders
13. Current Status of Gene Therapy
14. Gene Editing

2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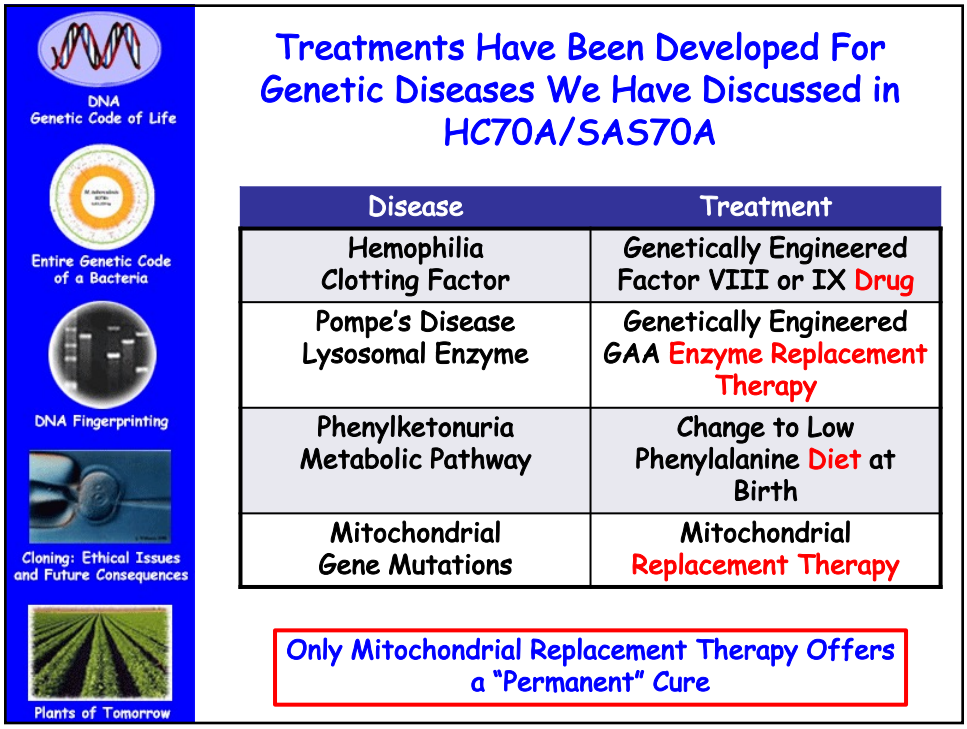
5 YEARS OMIM Human Genetics Knowledge for the World
OMIM®
Online Mendelian Inheritance in Man®
An Online Catalog of Human Genes and Genetic Disorders
5 YEARS OMIM Human Genetics Knowledge for the World
OMIM Morbid Map Scorecard (Updated February 24th, 2023) :
Total number of phenotypes* for which the molecular basis is known 7,346
Total number of genes with phenotype-causing mutation 4,765
Most Disease Genes Are Autosomal Recessive
Fewer Are Sex-Linked or Y-Linked or Mitochondrial
Disorder prevalence (approximate)
Autosomal dominant
Familial hypercholesterolemia 1 in 500[9]
Polycystic kidney disease 1 in 1250
Neurofibromatosis type I 1 in 2,500[10]
Hereditary spherocytosis 1 in 5,000
Marfan syndrome 1 in 4,000[11]
Huntington's disease 1 in 15,000[12]
Autosomal recessive
Sickle cell anaemia 1 in 625[13]
Cystic fibrosis 1 in 2,000
Tay–Sachs disease 1 in 3,000
Phenylketonuria 1 in 12,000
Mucopolysaccharidoses 1 in 25,000
Lysosomal acid lipase deficiency 1 in 40,000
Glycogen storage diseases 1 in 50,000
Galactosemia 1 in 57,000
X-linked
Duchenne muscular dystrophy 1 in 7,000
Hemophilia 1 in 10,000
Values are for liveborn infants

3


DNA
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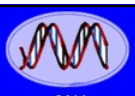
Plants of Tomorrow
Treatments Have Been Developed For Genetic Diseases We Have Discussed in HC70A/SAS70A
Disease
Treatment
Hemophilia Clotting Factor
Genetically Engineered Factor VIII or IX Drug
Pompe's Disease Lysosomal Enzyme
Genetically Engineered GAA Enzyme Replacement Therapy
Phenylketonuria Metabolic Pathway
Change to Low Phenylalanine Diet at Birth
Mitochondrial Gene Mutations
Mitochondrial Replacement Therapy
Only Mitochondrial Replacement Therapy Offers a "Permanent" Cure

4



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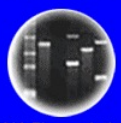

DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

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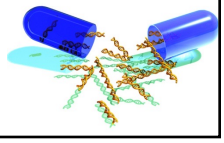
What Are the Prospects For a Permanent "Cure" For Genetic Diseases Using Gene Therapy?

5

What is Gene Therapy?

- 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*

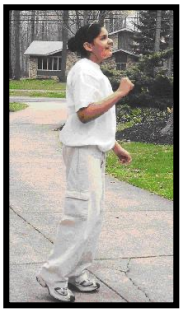


6

Humans Have Been Genetically Engineered to Cure Genetic Diseases

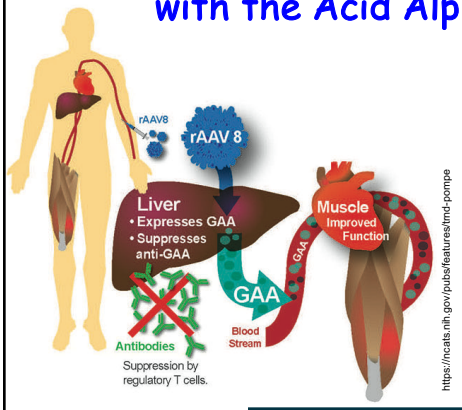







7

Gene Therapy for Pompe Disease with the Acid Alpha Glucosidase Gene



Clinical Trials for Pompe Disease in Progress

pompediseasenews.com

Investigational gene therapy	Status
AT845	In clinical development (Phase 1/2)
ACT-101	In clinical development (Phase 1/2)
SPK-3006	In clinical development (Phase 1/2)
rAAV1-CMV-hGAA	In clinical development (Phase 1/2)
rAAV9-DES-hGAA	In clinical development (Phase 1)
AVR-RD-03	In preclinical development

8

Gene Therapies for Hemophilia A & B

blood® Review Series

blood® 31 JANUARY 2019 | VOLUME 133, NUMBER 5 407

NEW THERAPEUTICS FOR INHERITED AND ACQUIRED BLEEDING CONDITIONS

Update on clinical gene therapy for hemophilia

George Q. Perrin,[1] Roland W. Herzog,[1,2] and David M. Markusic[2]

[1]Department of Pediatrics, Division of Cellular and Molecular Therapy, University of Florida, Gainesville, FL; and [2]Department of Pediatrics, Herman B Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, IN

FDA NEWS RELEASE

FDA Approves First Gene Therapy to Treat Adults with Hemophilia B

HEMGENIX® etranacogene dezaparvovec -XXXX

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For Immediate Release: November 22, 2022

News > Pharma News > FDA's decision on gene therapy Roctavian for adults with haemophilia A in early 2023

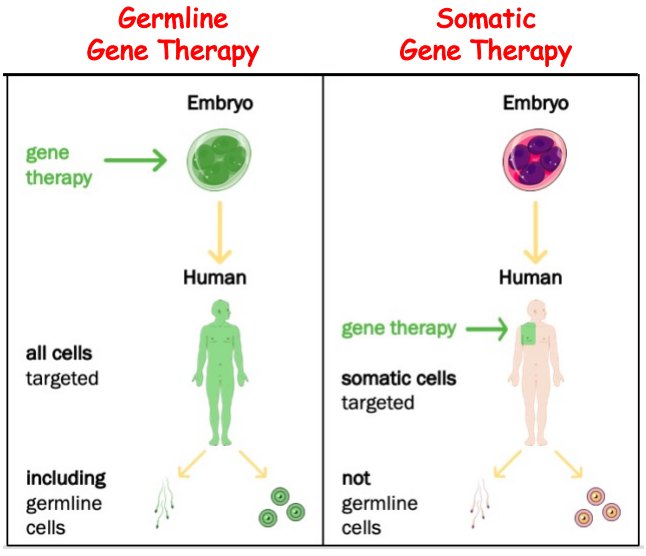
FDA'S DECISION ON GENE THERAPY ROCTAVIAN FOR ADULTS WITH HAEMOPHILIA A IN EARLY 2023

November 2022 | **PHARMA NEWS** | Nalinee Pathak

ROCTAVIAN® (Valoctocogen Roxaparvovec) Fertiglösung zur intravenösen Infusion

9

Gene Therapy Strategies

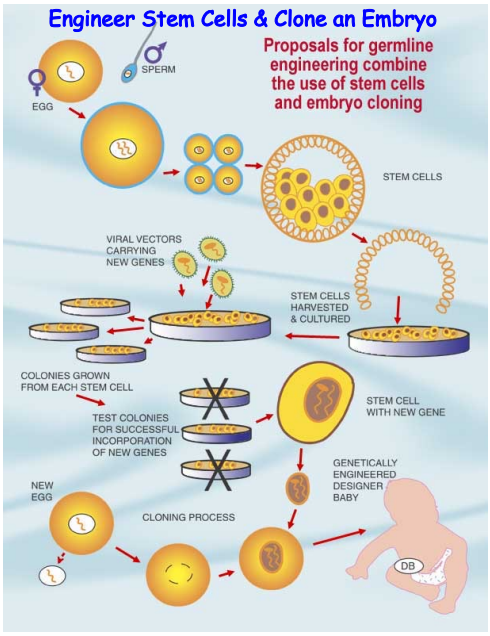


A somatic cell is any cell of a living organism other than the reproductive cells

10

Germline Gene Therapy

Germline gene therapy is the transfer of DNA 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



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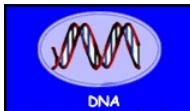
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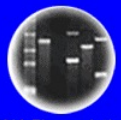

DNA Fingerprinting


Cloning: Ethical Issues and Future Consequences


Plants of Tomorrow

Road Blocks

Dickey-Wiker Amendment-1995

Federal Funds Cannot Be Used To:

- Create Human Embryos For Research Purposes
- Fund Research in Which a Human Embryo Will Be Destroyed, Discarded, or Knowingly Subjected to Risk or Injury of Death

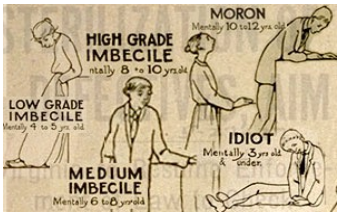
2023 Congressional Budget (Expires 9/30/23)

- FDA Cannot Spend Any Money to Review Applications For Clinical Trials That Involve Human Embryos With Heritable Genetic Modifications *(But…Male Mt Replacement Not Inherited & Egg Spindle Transfer Doesn't Destroy Embryo)*

12

Germline Gene Therapy is a "Slippery Slope" and Can Lead to Eugenics – There Are Many Ethical Issues

Some people are born to be a burden on the rest.




STEP TOWARD A SUPER-RACE

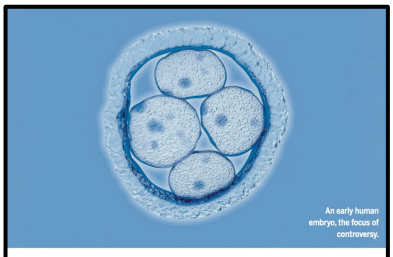
Health Official Praise Court in Upholding Sterilization Law

WASHINGTON, May 8.—(INS)—An improved race of Americans is on its

- Should Germline Gene Therapy Be Used to Correct Genetic Diseases?
- If So, Which Ones and Under What Circumstances?
- Is the Procedure Safe and Cause No Problems Throughout the Persons Entire Life?
- Should Germline Gene Therapy Be Used For Genetic Enhancement?
- If So, Which Traits?
- Will Changing the Human Genome Permanently Have Unintended Consequences to Human Populations in Future Generations?
- How will Germline Gene Therapy Be Regulated and By Whom?

13

Germline Gene Therapy Has Been Used in Humans!


An early human embryo, the focus of controversy.

BIOETHICS

Embryo engineering alarm

Researchers call for restraint in genome editing

Scientists Seek Ban on Method of Editing the Human Genome

By NICHOLAS WADE MARCH 19, 2015

A group of leading biologists on Thursday called for a worldwide moratorium on use of a new genome-editing technique that would alter human DNA in a way that can be inherited.

NEWS FEATURE | 26 February 2019 | Clarification 11 March 2019

The CRISPR-baby scandal: what's next for human gene-editing

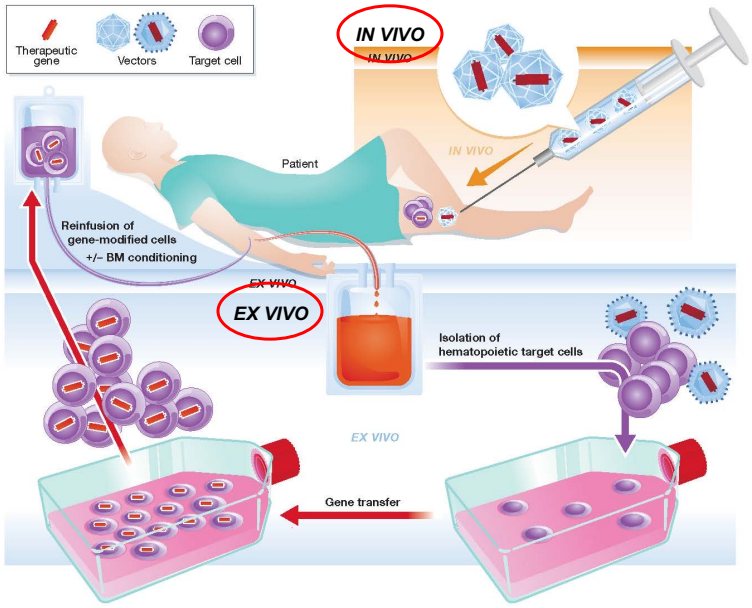
As concerns surge after a bombshell revelation, here are four questions about this fast-moving field.

Genome-edited baby claim provokes international outcry

Chinese Scientist Who Genetically Edited Babies Gets 3 Years in Prison

14

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



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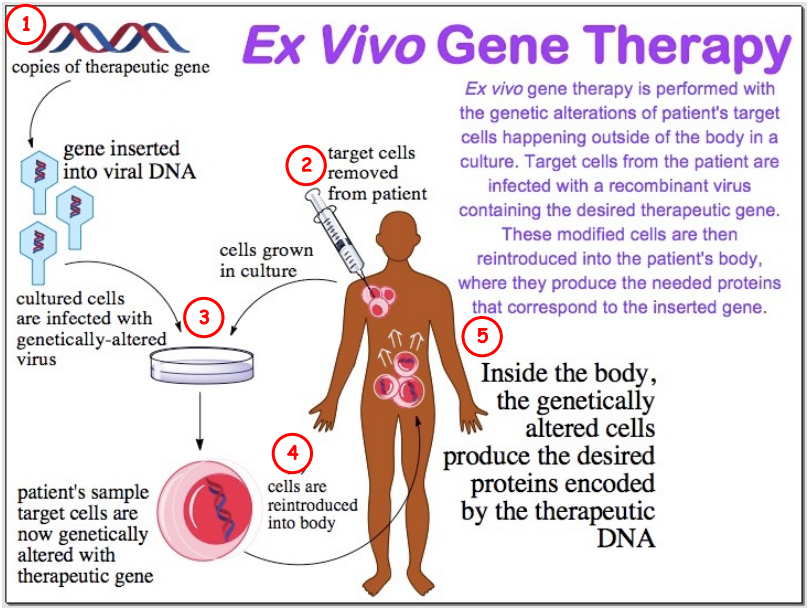


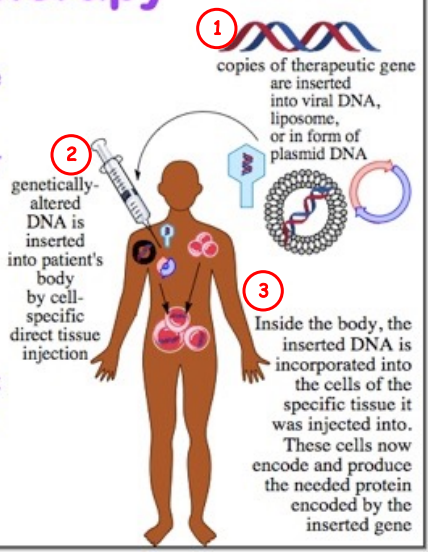
Ex Vivo Gene Therapy

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.

16

In Vivo Gene Therapy

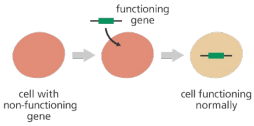
In vivo gene therapy involves introduction of therapeutic DNA directly into the patient's body. The DNA is introduced by cell-specific direct injection into tissue in need. DNA in the form of a plasmid vector is introduced by a dermal vaccination. Modified liposomes are not currently used for gene therapy,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



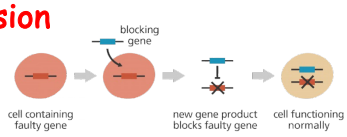
17

Somatic Gene Therapy Strategies

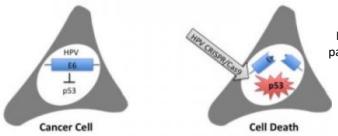
- **Gene augmentation**
  - Treat recessive genetic diseases



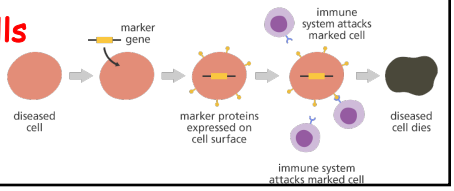
- **Targeted silencing of gene expression**
  - Treat dominant genetic diseases



- **Gene editing or replacement**
  - Treat recessive or dominant genetic diseases



- **Targeted killing of specific cells**
  - Treat cancers



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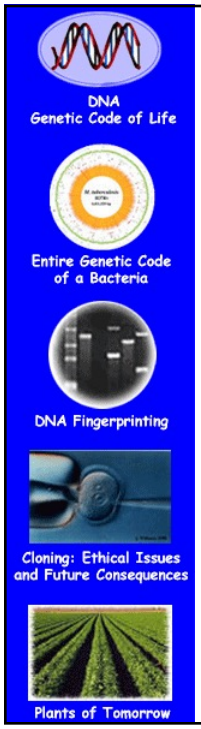
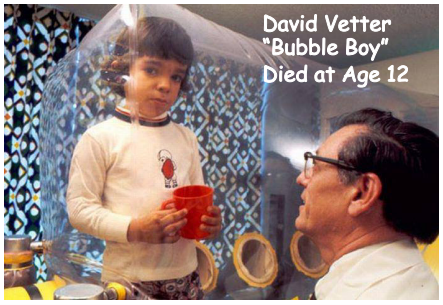


Ex Vivo Gene Therapy A Case Study for Severe Combined Immunodeficiency (SCID)

19

Severe Combined Immunodeficiency Diseases (SCID)

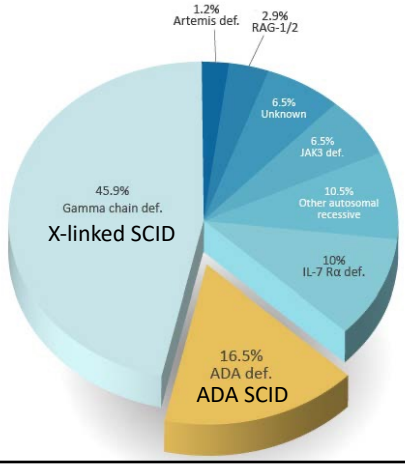


A group of rare, usually fatal unless treated, congenital disorders characterized by little or no immune response.

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

Relative Frequency of the Different Molecular Defects in SCID

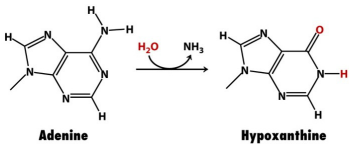


20

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

- ADA is an enzyme that degrades 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 (Enzyme Replacement Therapy – ERT)

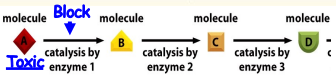
- 32,213 kb Gene
- Chromosome 20
- 12 Exons
- 1,092 kb mRNA
- 323 aa protein



ADA Causes the Degradation of Adenosine

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



21

Consider Two Questions

What Information is Needed Before Initiating Development of a Gene Therapy?

What "Tools" are Needed for Ex Vivo Somatic Cell Gene Therapy Protocols?

22

What Information is Needed Before Initiating Development of a Gene Therapy?

1.

2.

3.

4.

5.

23

What Information is Needed Before Initiating Development of a 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?

http://learn.genetics.utah.edu

24

What "Tools" are Needed for Ex Vivo Somatic Cell Gene Therapy Protocols?

1.

2.

3.

4.

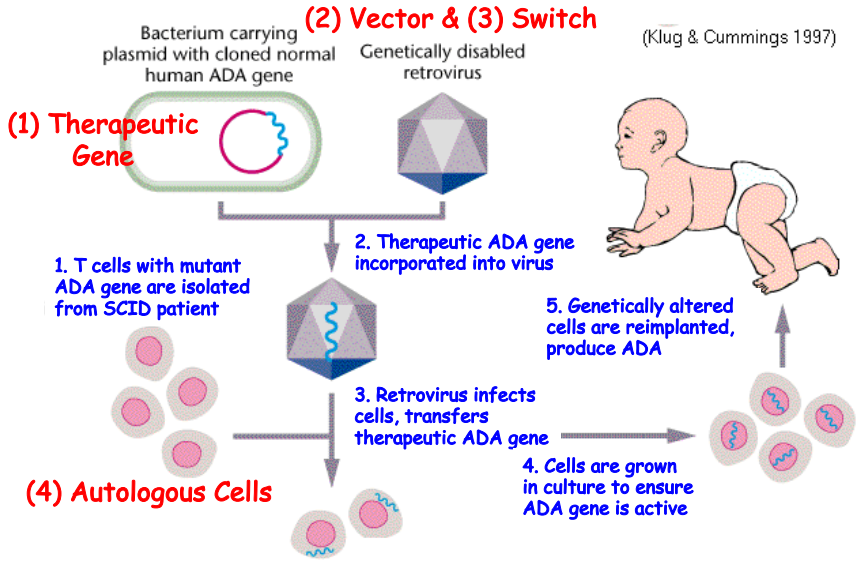
25

What "Tools" are Needed for Ex Vivo Somatic Cell Gene Therapy Protocols?

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

26

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



ADA-SCID Clinical Trial Started in 1990

27

Viral Vectors Used to Deliver Genes to Cells in Somatic Cell Gene Therapy

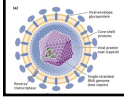
Table 1 Viral vectors discussed in this review

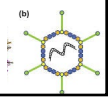
Features	Retroviral	Lentiviral	Adenoviral	AAV
Viral genome	RNA	RNA	DNA	DNA
Cell division requirement for target cell	Yes Yes	G1 phase No	No	No
Packaging limitation	8 kb	8 kb	8–30 kb	5 kb
Immune responses to vector	Few	Few	Extensive	Few
Genome integration	Yes	Yes	Poor	Poor
Long-term expression	Yes	Yes	No	Yes
Main advantages	Persistent gene transfer in dividing cells	Persistent gene transfer in transduced tissues	Highly effective in transducing various tissues	Elicits few inflammatory responses, nonpathogenic

Vectors Used in 2021 → Ex Vivo In Vivo

Natural Process – High Efficiency
Similar to Bacteriophages and *Agrobacterium*

How do You Use Viruses to Transfer the Therapeutic Gene into Cells?





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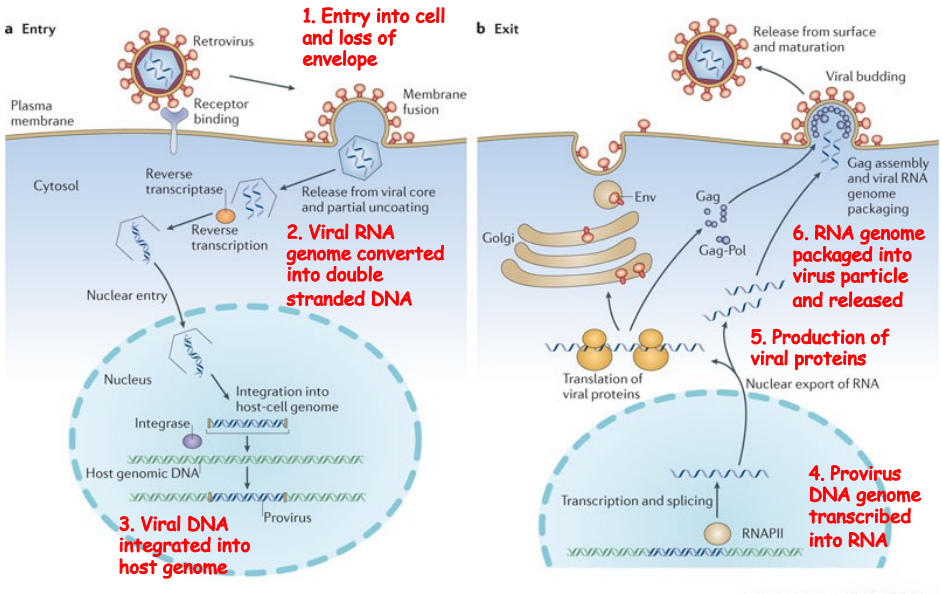
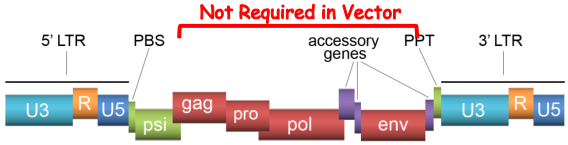
Retrovirus Life Cycle



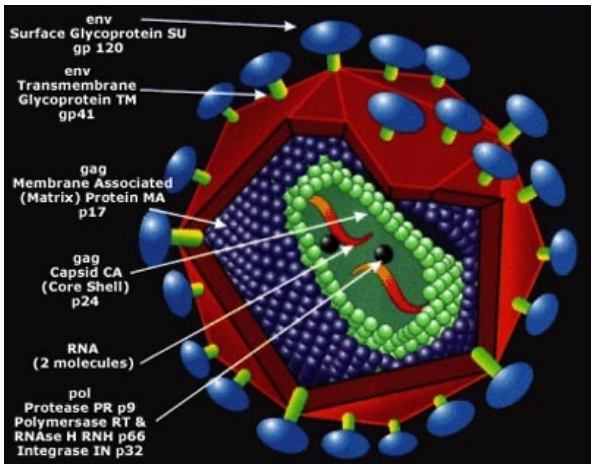
Retroviruses Replicate Using Reverse Transcriptase
David Baltimore & Howard Temin-Nobel Prize 1975

29

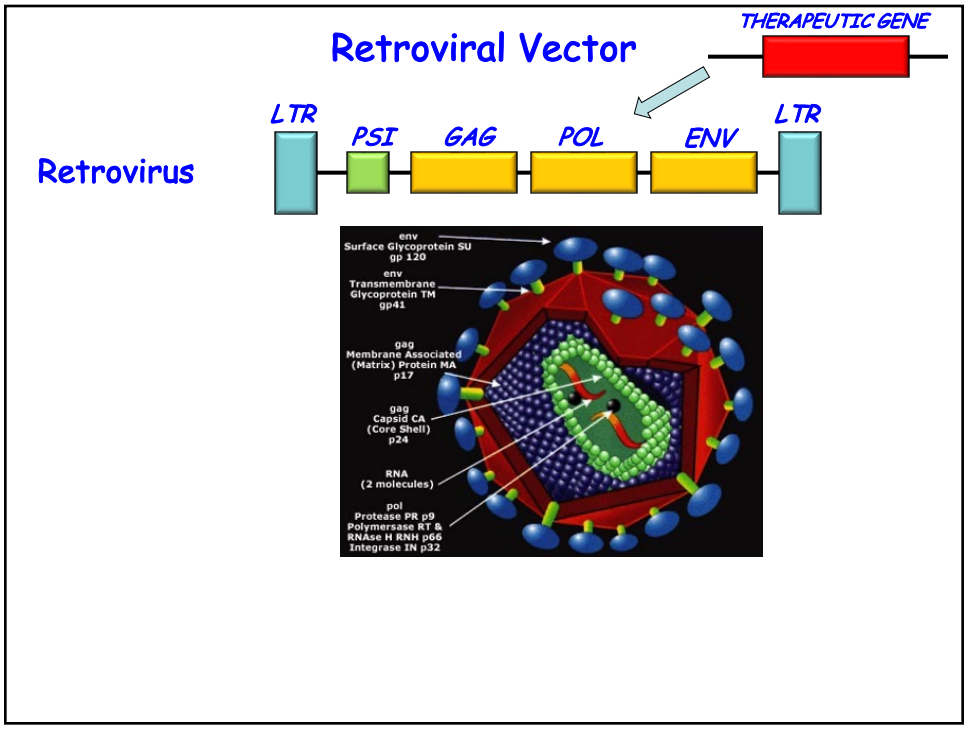
Retrovirus Genome



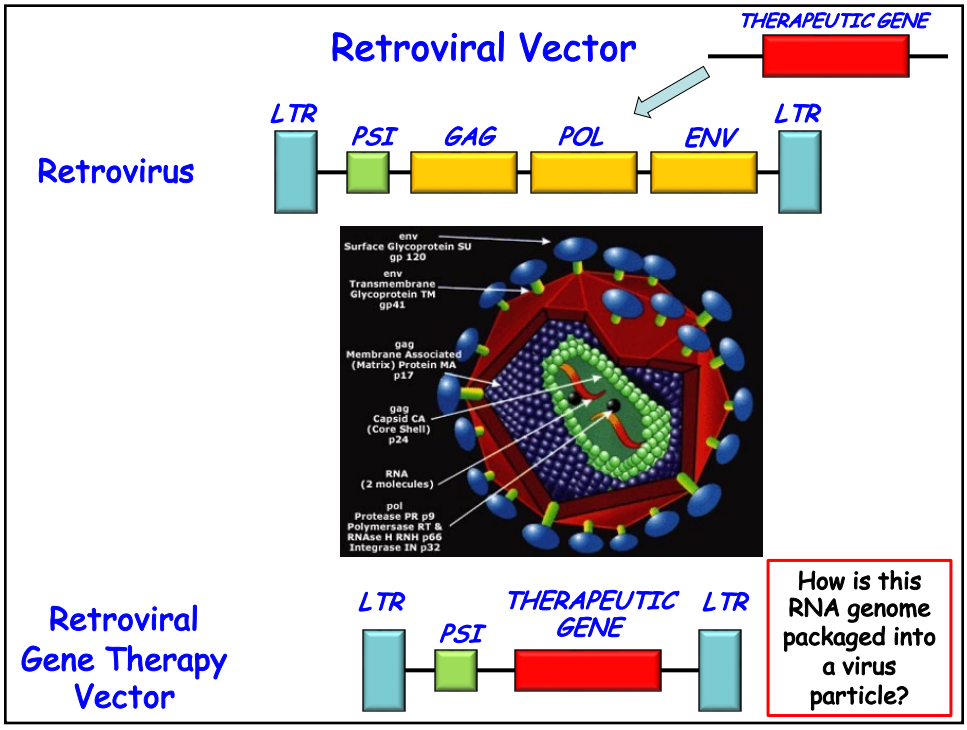
- 5' long terminal repeat (LTR) – strong switch & integration
- 3' LTR – strong switch, integration & transcriptional termination
- psi (Ψ) – packaging element needed to package the RNA genome into the viral particle
- gag – structural (coat) proteins
- pro – protease
- pol – reverse transcriptase
-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



30

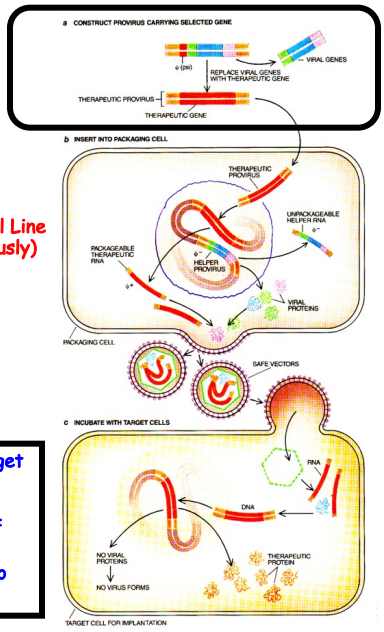

Retroviral Vector
THERAPEUTIC GENE
Retrovirus
LTR
PSI
GAG
POL
ENV
LTR
env
Surface Glycoprotein SU
gp 120
env
Transmembrane
Glycoprotein TM
gp41
gag
Membrane Associated
(Matrix) Protein MA
p17
gag
Capsid CA
(Core Shell)
p24
RNA
(2 molecules)
pol
Protease PR p9
Polymersase RT &
RNAse H RNH p66
Integrase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

31


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
LTR
PSI
THERAPEUTIC
GENE
LTR
How is this RNA genome packaged into a virus particle?

32

Using Retroviruses for Ex Vivo Gene Therapy



Packaging Cell Line (Made Previously)

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

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

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

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

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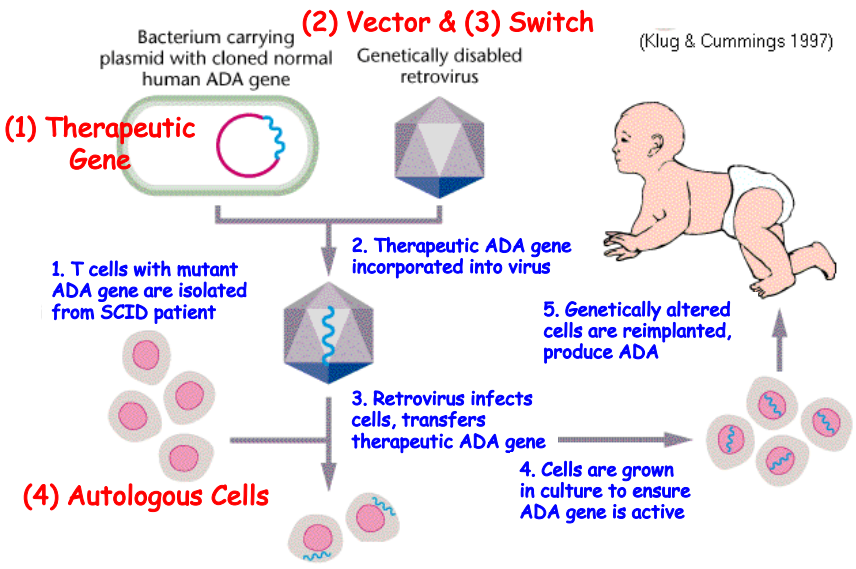
Ex Vivo Gene Therapy for ADA-Severe Combined Immunodeficiency (SCID)



ADA-SCID Clinical Trial Started in 1990

34

Did the Gene Therapy Strategy Work?

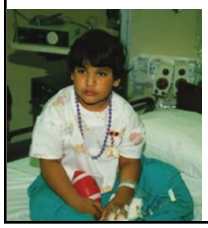
Science
AAAS

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
1992

Ashanthi DeSilva
2018

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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
- Human error - failure to adhere to strict NIH and IRB procedures (experimental therapies)
- Incomplete understanding of disease biology
- Insertional mutagenesis causing other diseases (e.g., leukemia)

The New York Times 1999

The Biotech Death of Jesse Gelsinger

By Sheryl Gay Stolberg
Published: November 28, 1999

Gelsinger died while undergoing gene therapy of systemic inflammatory response syndrome – an immune reaction to the adenovirus vector

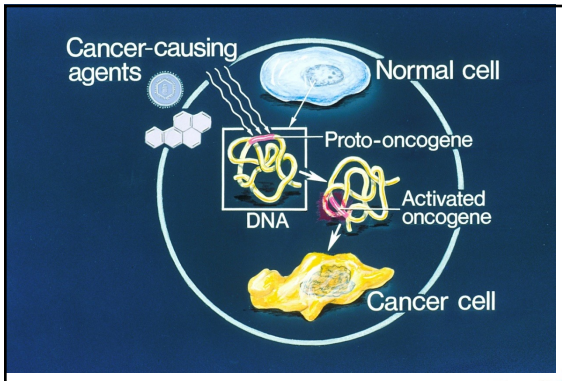
The New York Times 2002

TRIALS ARE HALTED ON A GENE THERAPY

By SHERYL GAY STOLBERG
Published: October 4, 2002

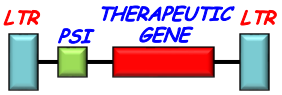
3 of 17 patients in clinical trial for X-SCID gene therapy developed clonal lymphoproliferative disorder – a leukemia

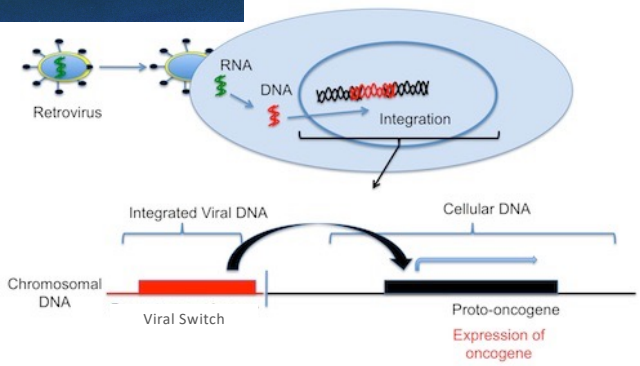
36



Retroviral Switches Can Activate Proto-Oncogenes and Induce Cancers

A Typical Retrovirus Gene Therapy Vector





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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 and Use of New And Safer Vectors
  - Lentivirus
  - Adeno Associated Virus (AAV)

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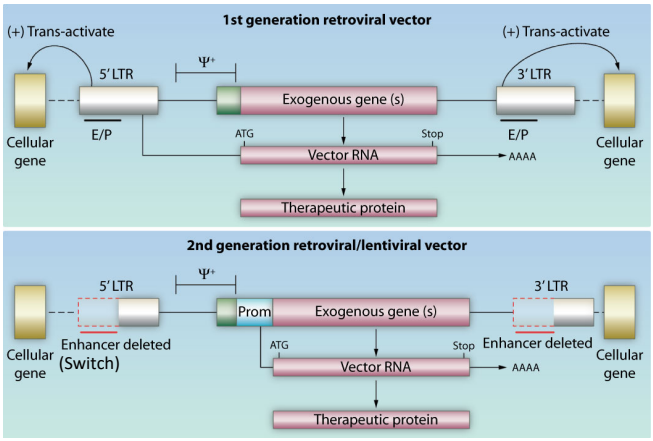
Development of Self-Inactivating (SIN) Vectors



1. First generation vectors often caused leukemia because they inserted viral DNA next to proto oncogenes (cancer causing genes).
2. The 5′ and 3′ LTRs of the viral vector are powerful switches 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.

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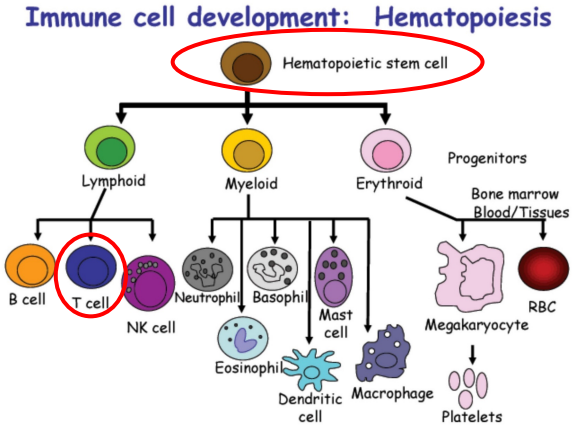
Using Stem Cell for *Ex Vivo* Gene Therapy



Dr. Pei-Yun Lee

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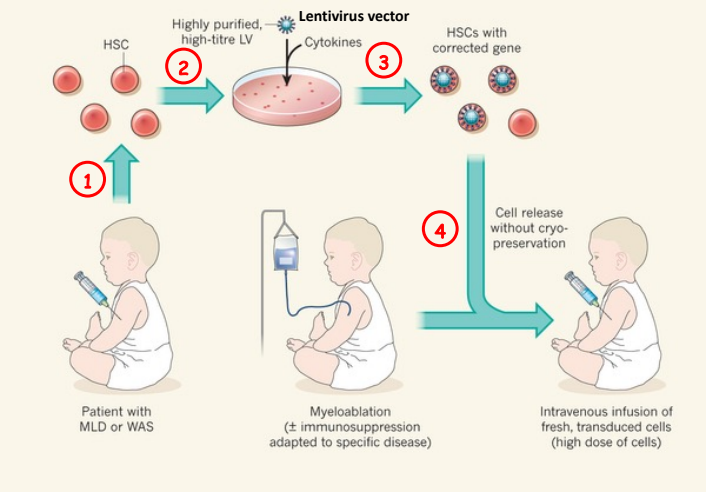
General Strategy for Use of Hematopoietic Stem Cells (HSCs) in Gene Therapy



1. Harvest stem cells from patient.
2. Culture and enrich for stem cells
3. Use lentivirus to transfer therapeutic gene into stem cells
4. Transfuse stem cell with corrected gene back into patient

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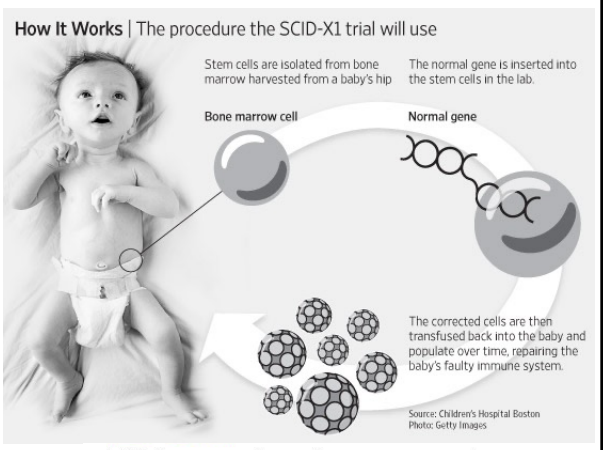
Updated Ex Vivo Gene Therapy for ADA-SCID & SCID-X1

- 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 Don Kohn

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

Peter Bracke | November 20, 2014

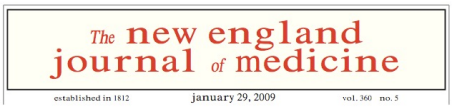
UCLA

43

It Works!

Gene therapy cures 'bubble boy disease'

31 Jan 2009, 1128 hrs IST, AP



Gene Therapy for Immunodeficiency Due to Adenosine Deaminase Deficiency

Results after 10 years

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

- ADA-SCID gene therapy product named Strimvelis from GlaxoSmithKline (sold to Orchard Therapeutics)
- Approved for use in Europe in May 2016, first used March 2017 - one time treatment costs $714,000, with money-back guarantee
- There is currently no FDA approved SCID gene therapy

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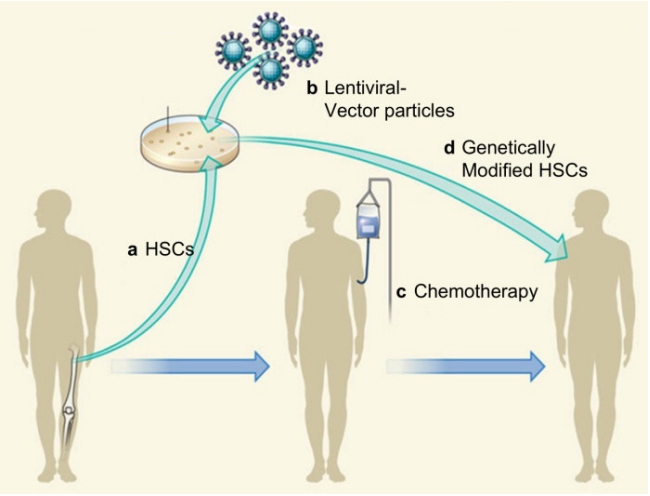
Many Diseases that are Being Targeted Using Ex Vivo Gene Therapy with Hematopoietic Stem Cells



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

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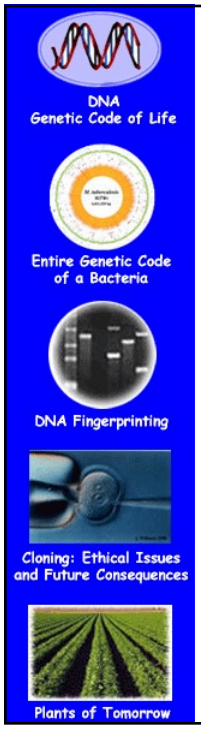

Ex Vivo Gene Therapy to Control Cancers

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The New York Times

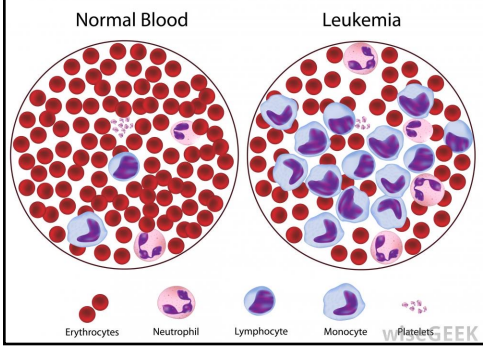
December 9, 2012

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

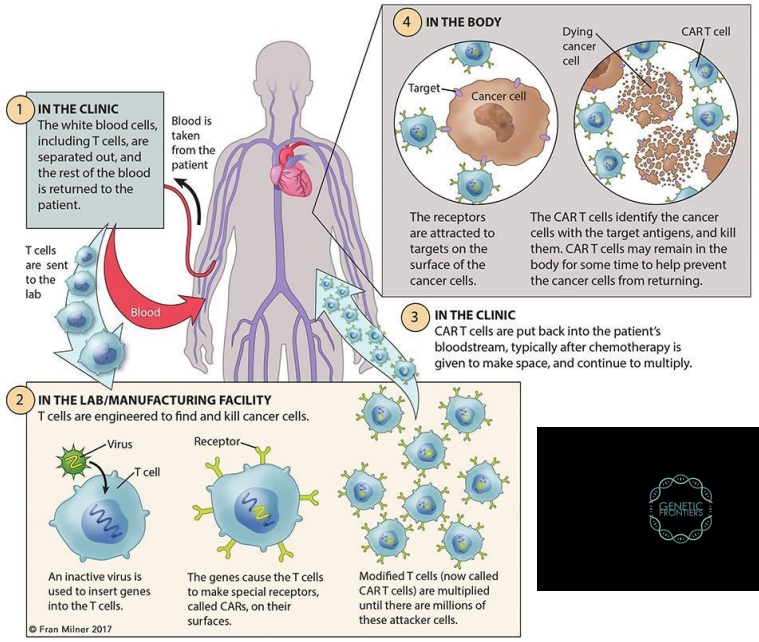


Emily Whitehead, 7, was the first child to receive gene therapy for leukemia at CHOP. (Photo courtesy of The Children's Hospital of Philadelphia)

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

47

Chimeric Antigen Receptor (CAR) T Cell Strategy



https://www.youtube.com/watch?v=mXADrg_ckhI

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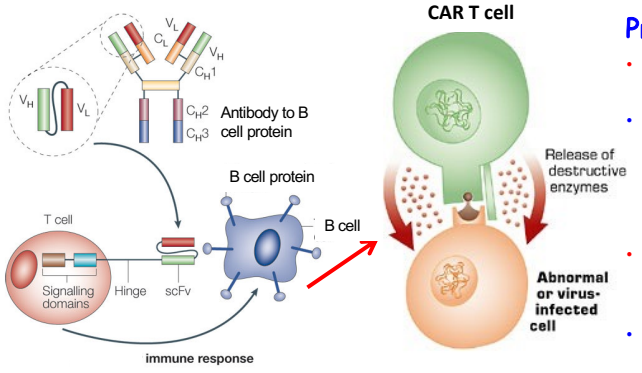
Ex-vivo Gene Therapy for Lymphocytic Leukemia

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

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FDA-Approved Car-T Cell Gene Therapies

(as of 12/16/2022)

BRAND NAME	GENERIC NAME	TARGETED DISEASE	FDA Approval
Kymriah™	tisagenlecleucel	Follicular Lymphoma, Diffuse Large B-cell Lymphoma, or Lymphoblastic Leukemia	2017
Yescarta™	axicabtagene ciloleucel	Follicular Lymphoma or Diffuse Large B-cell Lymphoma	2017
Tecartus™	brexucabtagene autoleucel	Mantle Cell Lymphoma or Acute Lymphoblastic Leukemia	2021
Breyanzi®	lisocabtagene maraleucel	Large B-cell Lymphoma	2022
Abecma®	idecabtagene vicleucel	Relapsed or Refractory Multiple Myeloma	2021
Carvykti™	ciltacabtagene autoleucel	Relapsed or Refractory Multiple Myeloma	2022
Provenge	sipuleucel-T	Prostate Cancer	2022

OriGen BIOMEDICAL

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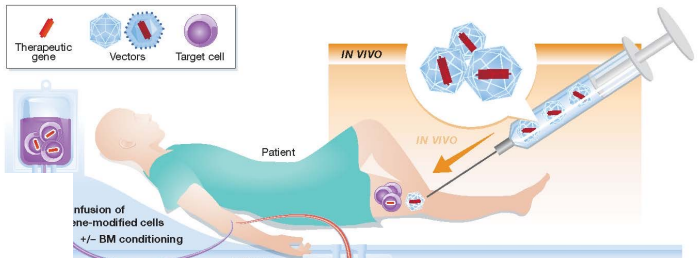
In Vivo Gene Therapy



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What "Tools" are Needed for ~~Ex Vivo~~ In Vivo Somatic Cell Gene Therapy Protocols?

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. *Ability to target the vector to desired cells*
   ~~Autologous cells (obtained from the same individual) or non-autologous cells~~

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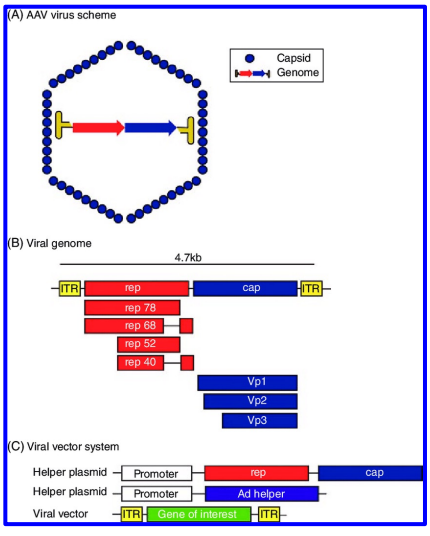
Adeno-Associated (AAV) Viruses have a 5kb Single Stranded Genome and are Excellent Vectors For *In Vivo* Gene Therapy



Replacement of Defective Recessive Genes

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Adeno-Associated Viruses Infect a Wide Range of Dividing and Non-Dividing Cell Types……

Primary Target Tissues									
Serotype	Retina	Neurons	Brain	Lung	Heart	Liver	Muscle	kidney	Pancreas
AAV-1		√			√		√		√
AAV-2	√	√	√			√	√	√	
AAV-3	√			√		√	√		
AAV-4	√	√	√				√		
AAV-5	√	√		√					
AAV-6				√	√	√	√		
AAV-7	√	√				√	√		√
AAV-8	√		√			√	√		
AAV-9			√	√	√	√	√	√	√
AAV-10		√		√	√	√	√		
AAV-DJ	Efficiently transduces a wide variety of cell types *in vitro*								
AAV-DJ/8	A variant of AAV-DJ that permits infection of liver as well as other tissues *in vivo*								

…..Making Them the "Favorite" Vector for Gene Delivery to Many Different Organs

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Many Different Genetic Diseases are Being Treated with *In Vivo* Gene Therapy

Primary gene delivery target	Condition	AAV capsid	Transgene product	Strategy	Sponsor	Phase	ClinicalTrials.gov identifier
Liver	Haemophilia B	AAV8	FIX	Replacement	Shire	Phase I/II	NCT01687608
		ND	FIX	Replacement	Pfizer	Phase II	NCT02484092
		ND	FIX	Replacement	Pfizer	Phase III	NCT03587116
		AAV6	FIX	Replacement	Sangamo	Phase I	NCT02695160
		AAV8	FIX	Replacement	St. Jude Children's Research Hospital	Phase I	NCT00979238
		AAV5	FIX	Replacement	uniQure	Phase III	NCT03569891
		ND	FIX	Replacement	UCL	Phase I	NCT03369444
	MPS-I	AAV6	ZFN1, ZFN2 and IDUA donor	Editing	Sangamo	Phase I	NCT02702115
	MPS-II	AAV6	ZFN1, ZFN2 and IDS donor	Editing	Sangamo	Phase I	NCT03041324
	MPS-IIIA	AAVrh.10	SGSH	Replacement	LYSOGENE	Phase II/III	NCT03612869
	MPS-VI	AAV8	ARSB	Replacement	Fondazione Telethon	Phase I/II	NCT03173521
	OTC deficiency	AAV8	OTC	Replacement	Ultragenyx	Phase I/II	NCT02991144
Muscle	A1AT deficiency	AAV2	A1AT	Replacement	UMMS	Phase I	NCT00377416
	CMT1A	AAV1	NTF3	Addition	Nationwide Children's Hospital	Phase I/II	NCT03520751
	DMD	AAVrh.74	Micro-dystrophin	Replacement	Nationwide Children's Hospital	Phase I/II	NCT03375164
		AAV9	Mini-dystrophin	Replacement	Pfizer	Phase I	NCT03362502
		AAV9	Micro-dystrophin	Replacement	Solid Biosciences	Phase I/II	NCT03368742
	Dysferlinopathy	AAVrh.74	DYSF	Replacement	Nationwide Children's Hospital	Phase I	NCT02710500
	HIV infections	AAV1	PG9 antibody	Addition	International AIDS Vaccine Initiative	Phase I	NCT01937455
		AAV8	VRC07 antibody	Addition	NIAID	Phase I	NCT03374202
	Pompe disease	AAV8	GAA	Replacement	Actus Therapeutics	Phase I/II	NCT03533673
		AAV9	GAA	Replacement	University of Florida	Phase I	NCT02240407
	X-linked MTM	AAV8	MTM1	Replacement	Audentes	Phase I/II	NCT03199469

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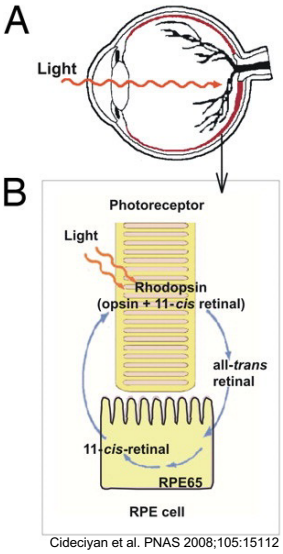
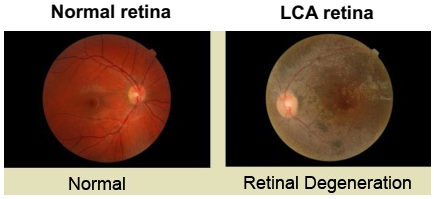
Blindness - Leber Congenital Amaurosis (LCA)

How We See

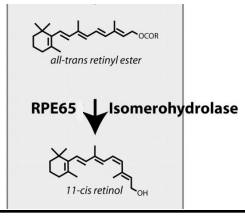


Cideciyan et al. PNAS 2008;105:15112

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



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



A Gene Therapy for LCA

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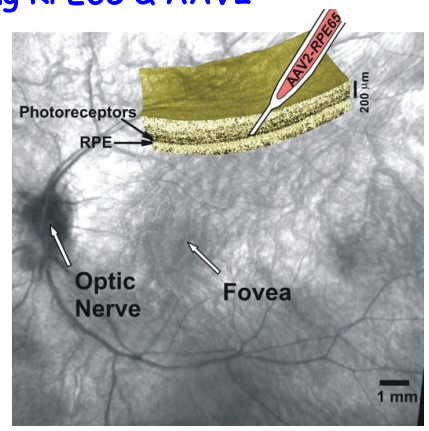
LCA Gene Therapy Using RPE65 & AAV2

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



LUXTURNA™
voretigene neparvovec-rzyl
for subretinal injection

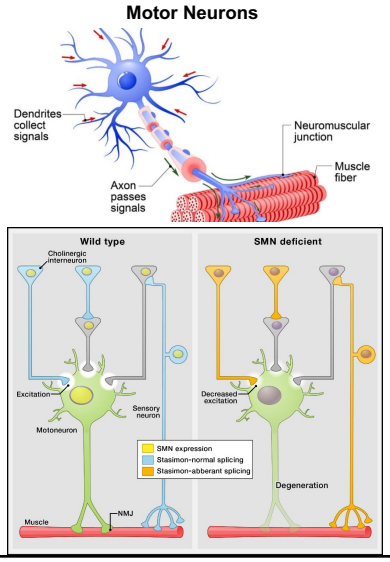
NOW A REALITY: THE FIRST FDA-APPROVED GENE THERAPY FOR A GENETIC DISEASE

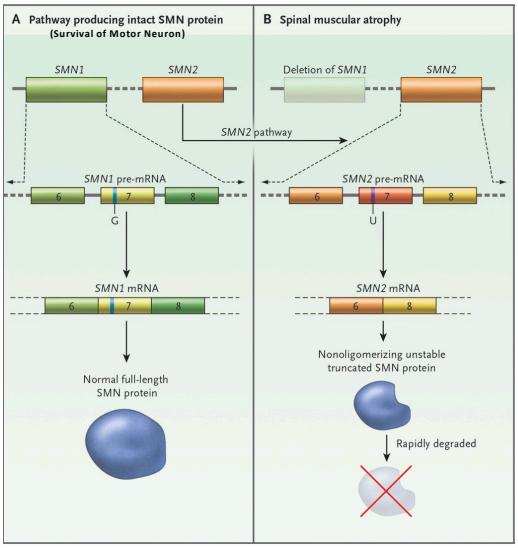
- FDA Approval December 19, 2017
- $425,000 per eye
- Money-back guarantee

57

Spinal Muscular Atrophy (SMA)

- Spinal Muscular Atrophy is an autosomal recessive neurodegenerative disease
- Number one genetic cause of infant mortality, with life expectancy of <2 years
- Characterized by progressive muscle weakness caused by a loss of specialized nerve cells (motor neurons) in the spinal cord and brainstem





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In-vivo Gene Therapy for SMA Type 1

Protocol for Phase 1 Clinical Trial

- Transferred the SMN1 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 treatment 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

zolgensma®
(onasemnogene abeparvovec-xioi)

FDA Approved Gene Therapy for SMA Type 1
$2.1M per dose – when approved in 2019, it was the most expensive drug in the world

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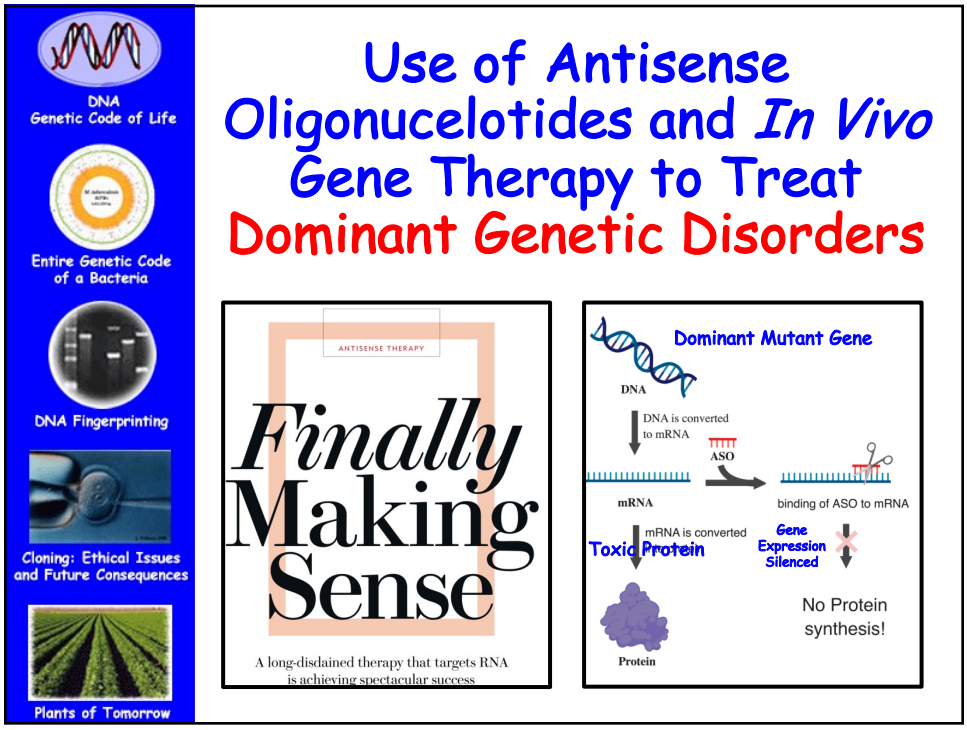

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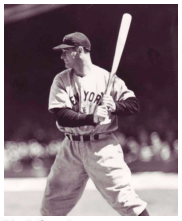
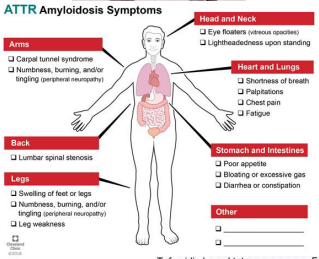
Examples of Dominant Genetic Diseases



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

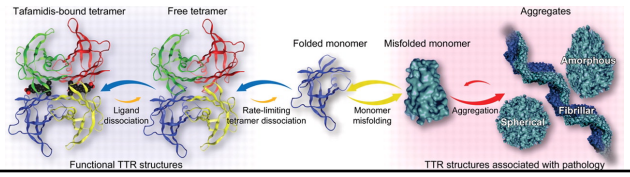
- One cause is a dominant mutation in the *superoxide dismutase (SOD1)* gene
- Mutant SOD1 Protein is Toxic to Motor Neurons



Hereditary Transthyretin (hATTR) Amyloidosis

- A dominant mutation in the *transthyretin (TTR)* gene causes the protein to fold abnormally.
- The abnormal TTR protein aggregates into amyloid fibrils in a number of organs, eventually causing death

Properly folded TTR protein is soluble



Improperly folded TTR protein forms aggregates

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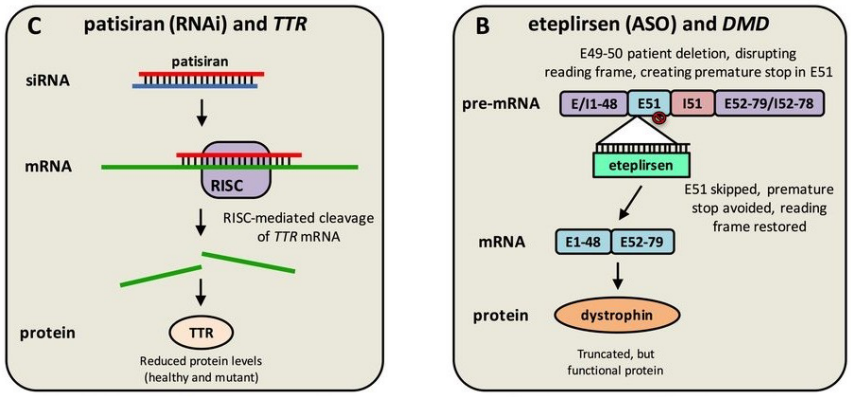
Antisense Oligonucleotides (ASOs) are Complementary to mRNA, and They Can Be Used to Degrade Specific mRNAs or Alter Splicing of Specific Pre-mRNAs



familial amyloid polyneuropathy

Duchenne muscular dystrophy

Allele-specific ASOs anneal to complementary mRNA, marking the mRNA for destruction.

Recessive normal allele can now function properly

Allele-specific ASOs anneal to complementary pre-mRNA, altering splicing, and resulting in the elimination of Exon 51.
The truncated protein can function properly.

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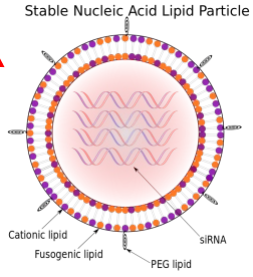
ASO Gene Therapy for Transthyretin-mediated Amyloidosis

- Protocol
  - Create an ASO against mutant transthyretin (TTR) mRNA with a modified phosphodiester RNA backbone
  - Encapsulate siRNA in lipid nanocarriers
  - Deliver the drug intravenously
- Results
  - Observed a 82 – 87% mean reduction in TTR levels
  - Efficiency of TTR knockdown supports monthly or bimonthly dosing
  - No adverse effects observed

Alnylam PHARMACEUTICALS
onpattro (patisiran) lipid complex injection

FDA Approved 2018

(Not classified as a gene therapy treatment – why not?)



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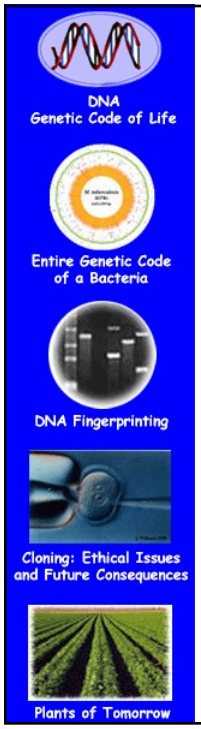

Current Status of Gene Therapy

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Timeline of Regulatory Authority for Gene Therapy in the USA

1974 — NIH becomes locus of rDNA research oversight; Recombinant DNA Advisory Committee (RAC) established

1975 — RAC develops biosafety guidelines after Asilomar Conference

1976 — *NIH Guidelines for Research Involving Recombinant DNA Molecules* published

1984 — FDA begins to regulate gene-therapy products

1989–1990 — NIH director approves first gene-therapy protocol under *NIH Guidelines*; first gene-therapy administration occurs

1991 — FDA issues first guidance document, *Points to Consider in Human Somatic Cell Therapy and Gene Therapy*

1997 — NIH eliminates director approval of individual protocols; FDA assumes sole authority to approve gene-therapy protocols

1999 — Death of Jesse Gelsinger, research participant in a gene-therapy clinical trial

2000 — FDA starts gene-therapy clinical trials monitoring plan to strengthen protections for trial participants

2000–2002 — NIH and FDA harmonize requirements for reporting serious adverse events; ClinicalTrials.gov launched

2003 — FDA issues temporary moratorium on use of retroviral vectors in blood stem cells because of risk of insertional mutagenesis resulting in malignancy

2014 — IOM report recommends limiting RAC review to exceptional protocols

2016 — NIH implements streamlining of protocol submission and review

2017 — FDA approves first gene-therapy products; revised Common Rule strengthens research participant protections

2018 — NIH and FDA propose elimination of unnecessary duplicative oversight; RAC to focus on emerging biotechnology issues; FDA draft guidance on gene therapy published

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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 (CBER) 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
    - CBER reviews and approves applications for gene therapy clinical trials
    - FDA cannot review applications for clinical trials that involve human embryos with heritable genetic modifications

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

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FDA Approved Gene Therapy Products

(12/16/22)

IMLYGIC® (talimogene laherparepvec) INTRALESIONAL INJECTION
Metastatic Melanoma 2015

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

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

KYMRIAH® 2017 (tisagenlecleucel) Suspension for IV infusion
CAR-T Therapy 2017

zolgensma® (onasemnogene abeparvovec-xioi)
Spinal Muscular Atrophy 2019

TECARTUS® (brexucabtagene autoleucel) Suspension for IV infusion
Car-T Therapy 2021

Abecma™ (idecabtagene vicleucel) SUSPENSION FOR IV INFUSION
Car-T Therapy 2021

zynteglo® (betibeglogene autotemcel) suspension for IV infusion
β Thalassemia 2022

HEMGENIX® etranacogene dezaparvovec -xxxx
Hemophilia B 2022

ADSTILADRIN
Bladder Cancer 2022

Breyanzi® (lisocabtagene maraleucel) SUSPENSION FOR IV INFUSION
Car-T Therapy 2022

CARVYKTI™ (ciltacabtagene autoleucel) Suspension for IV infusion
Car-T Therapy 2022

PROVENGE® (sipuleucel-T)
Car-T Therapy 2022

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Some Issues With Human Gene Therapy

- More Information is Needed
- Germline Gene Therapy
- Consent
- Eugenics & the "Slippery Slope" Towards Enhancement
- Availability for Everyone

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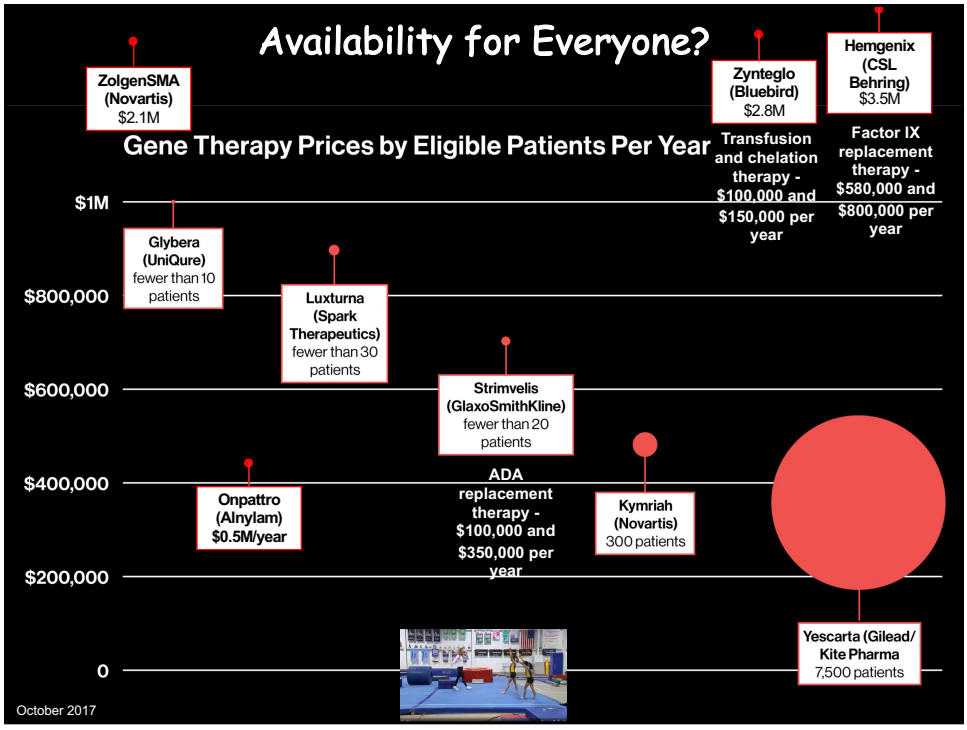

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DNA
Genetic Code of Life
Entire Genetic Code of a Bacteria
DNA Fingerprinting
Cloning: Ethical Issues and Future Consequences
Plants of Tomorrow
Gene Editing & Human Gene Therapy
Dominant & Recessive Genes
Germline & Somatic Cell Gene Therapy
Editing Does it All!
DNA editing
A DNA editing technique, called CRISPR/Cas9, works like a biological version of a word-processing programme's "find and replace" function.
HOW THE TECHNIQUE WORKS
Cell
Nucleus
Chromosome
Guide molecule
DNA-cutting enzyme
Defective DNA strand
Healthy DNA strand
A cell is transfected with an enzyme complex containing:
Guide molecule
Healthy DNA copy
DNA-cutting enzyme
A specially designed synthetic guide molecule finds the target DNA strand.
An enzyme cuts off the target DNA strand.
The defective DNA strand is replaced with a healthy copy.
Sources: Reuters; Nature; Massachusetts Institute of Technology

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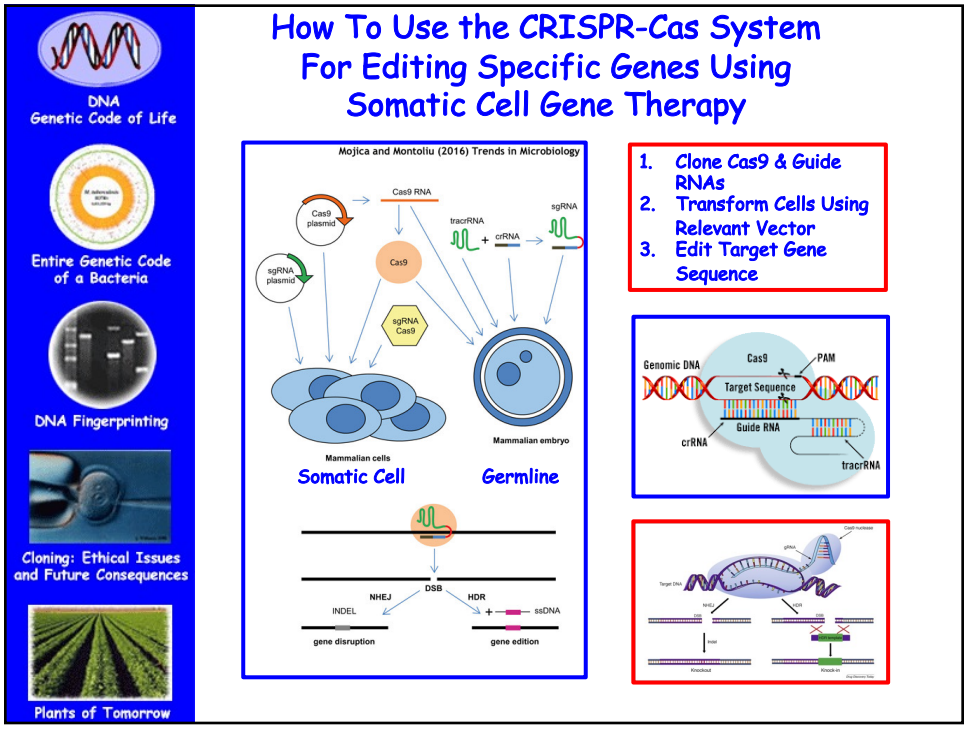
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How To Use the CRISPR-Cas System For Editing Specific Genes Using Somatic Cell Gene Therapy
Mojica and Montoliu (2016) Trends in Microbiology
Cas9 plasmid
Cas9 RNA
tracrRNA
crRNA
sgRNA
sgRNA plasmid
Cas9
sgRNA Cas9
Mammalian cells
Mammalian embryo
Somatic Cell
Germline
DSB
NHEJ
HDR
INDEL
ssDNA
gene disruption
gene edition
1. Clone Cas9 & Guide RNAs
2. Transform Cells Using Relevant Vector
3. Edit Target Gene Sequence
Genomic DNA
Cas9
PAM
Target Sequence
Guide RNA
crRNA
tracrRNA

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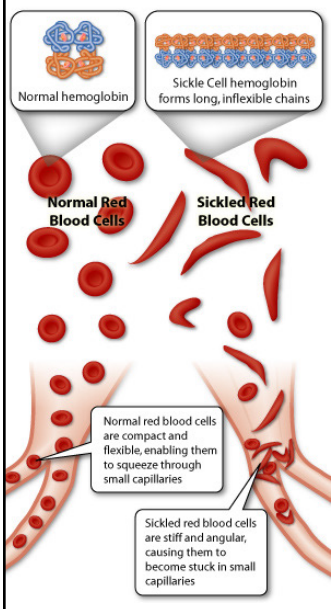
Sickle Cell Disease



- **Disease**
  - Sickled cells are rapidly destroyed, causing anemia and jaundice
  - Sickled cells block blood flow through vessels, potentially resulting in lung, spleen, kidneys, eyes and liver damage, pain, and strokes
- **Cause**
  - Recessive mutation results in a single amino acid change in the β subunit of hemoglobin (Hb)
  - HbS folds abnormally, forming HbS fibers that cause red blood cells to have a sickle shape
- **Current Therapies**
  - Blood transfusions
  - Hydroxyurea – decreases pain
  - Bone marrow (stem cell) transplant

https://www.youtube.com/watch?v=mQ8Ola_C5po&t=8s

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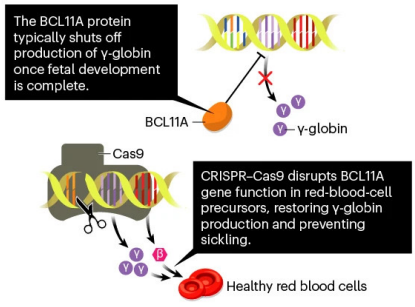
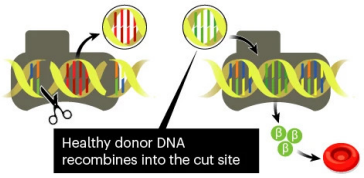
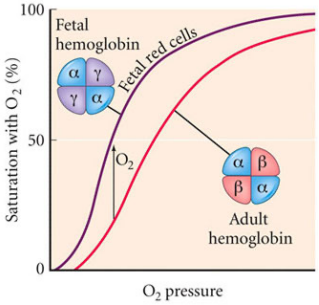
Sickle Cell Disease Gene Therapies

1. CRISPR Gene Editing Therapy to Activate Fetal Hb Gene in Adults



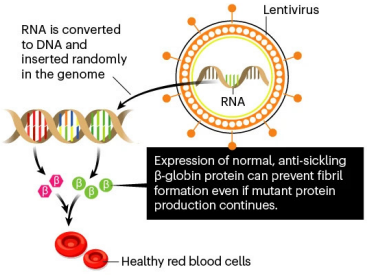
2. CRISPR Gene Editing Therapy to Repair the HbS Gene





Oxygen Binds More Fetal Hb Strongly than Adult Hb

3. Gene Augmentation Therapy to Replace the HbS Gene



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NIH U.S. National Library of Medicine
ClinicalTrials.gov

Phase 1/2/3 Gene Editing Therapy Clinical Trials Currently in Progress for these Diseases (02/28/23)

Phenylketonurias
B Cell Leukemia
Gastrointestinal Epithelial Cance
Duchenne Muscular Dystrophy
Severe Combined Immunodeficiencies (SCID)
Sickle Cell Disease
Carcinoma, Non-Small-Cell Lung
T Cell Lymphoma
Renal Cell Carcinoma
Transthyretin-Related (ATTR) Familial Amyloid Polyneuropathy
HIV-1-infection
Multiple Myeloma
Melanoma
Non-muscle-invasive Bladder Cancer
Pancreatic Cancer
Chronic Lymphocytic Leukemia
Transfusion Dependent Beta Thalassemia
Beta-Thalassemia
Leber Congenital Amaurosis 10

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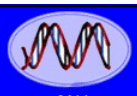

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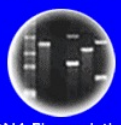
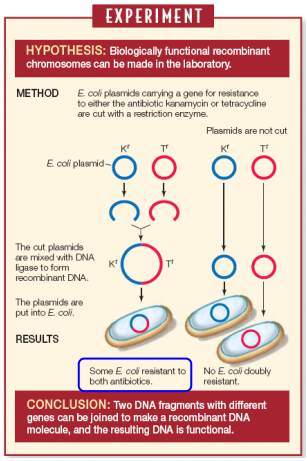


DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

The End!!
Lectures on the History, Science, and Applications of Genetic Engineering……



…….On to Genetic Engineering & the Law

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