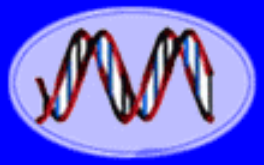

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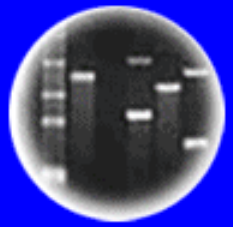


DNA Fingerprinting

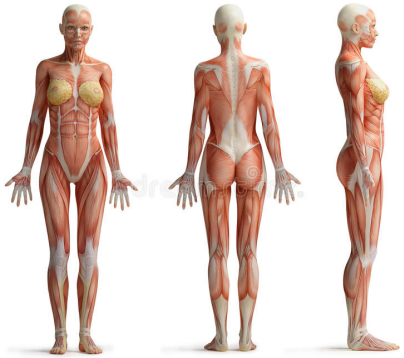
Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

HC70A & PLSS059
Winter 2020
Genetic Engineering in Medicine, Agriculture, and Law

Professors Bob Goldberg & Channapatna Prakash

Lecture 8
Human Genetic Engineering



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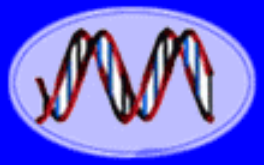

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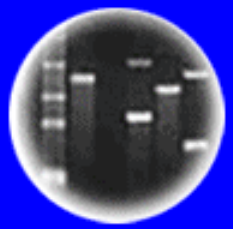

DNA Fingerprinting

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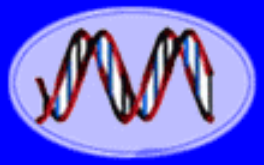
THEMES

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
3. Ex Vivo Gene Therapy for Cancer
4. *In Vivo* Gene Therapies
5. Regulation of Gene Therapy
6. Current Status of Gene Therapy
7. Issues Concerning Gene Therapy
8. Gene Editing & Human Gene Therapy



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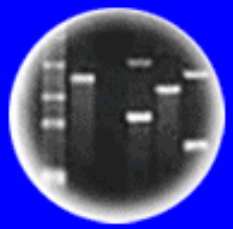

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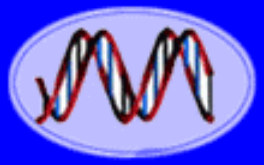
Recent Gene Therapy Reviews

Anguela. X.M. and High, K.A. (2019) Entering the Modern Era Of Gene Therapy. *Annual Review Medicine* 70, 273-288

Wang, D., Tai, P.W.L., and Gao, G. (2019) Adeno-associated Virus as a Platform for Gene Therapy Delivery. *Nature Reviews Drug Discovery* 18, 358-378.

Duodna, J. (2020) The Promise and Challenge of Therapeutic Gene Editing. *Nature* 578, 229-236.



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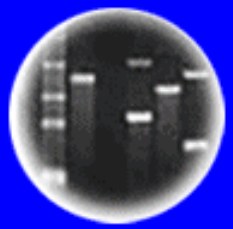

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5 YEARS OMIM
Human Genetics Knowledge for the World

OMIM®

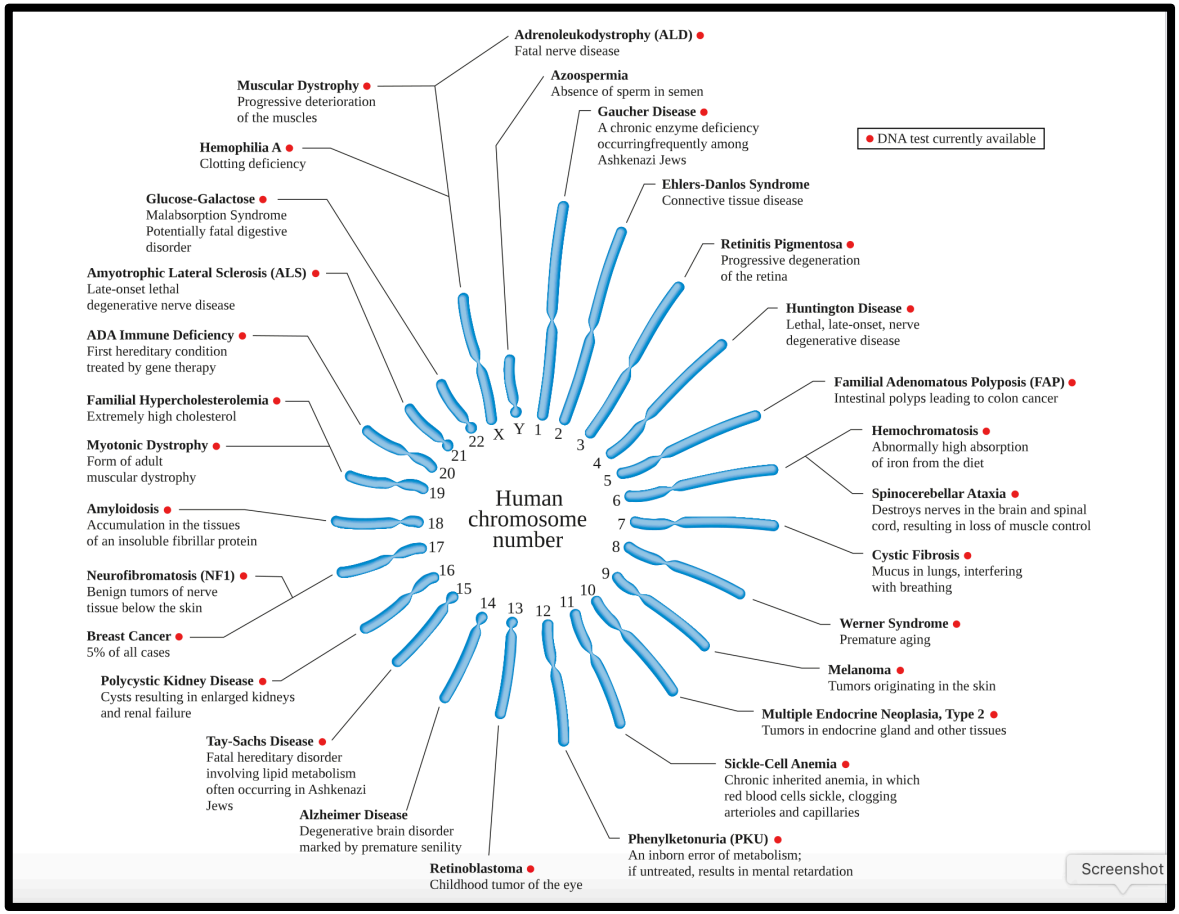
Online Mendelian Inheritance in Man®

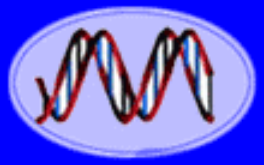
An Online Catalog of Human Genes and Genetic Disorders

5 YEARS OMIM
Human Genetics Knowledge for the World

OMIM Morbid Map Scorecard (Updated Febuary 21st, 2020) :

Total number of phenotypes* for which the molecular basis is known	6,601
Total number of genes with phenotype-causing mutation	4,229





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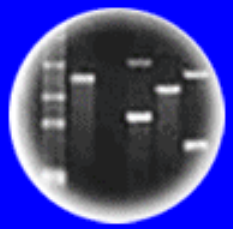

DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

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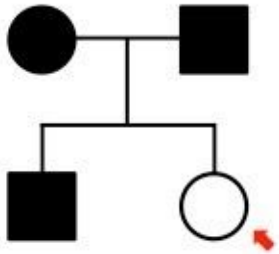
Family Pedigrees Determine Mode of Inheritance

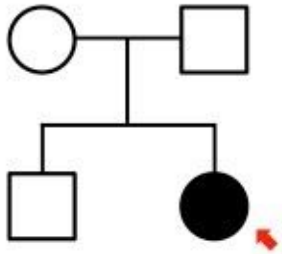
AUTOSOMAL DOMINANT



Cannot be recessive as two affected parents could **not** have an unaffected offspring

Parents MUST be heterozygous

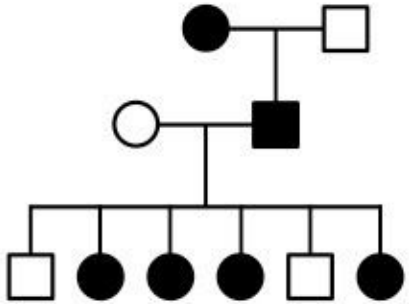
AUTOSOMAL RECESSIVE



Cannot be dominant as two unaffected parents could **not** have an affected offspring

Parents MUST be heterozygous

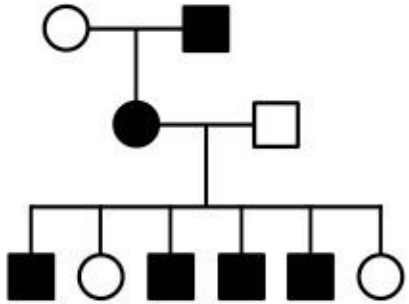
X-LINKED DOMINANT



Sex linkage cannot be confirmed

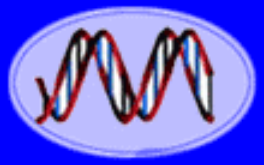
100% incidence of affected daughters from an affected father *suggests* X-linked dominance

X-LINKED RECESSIVE



Sex linkage cannot be confirmed

100% incidence of affected sons from an affected mother *suggests* X-linked recessive



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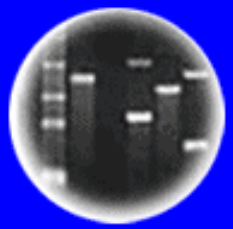

DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

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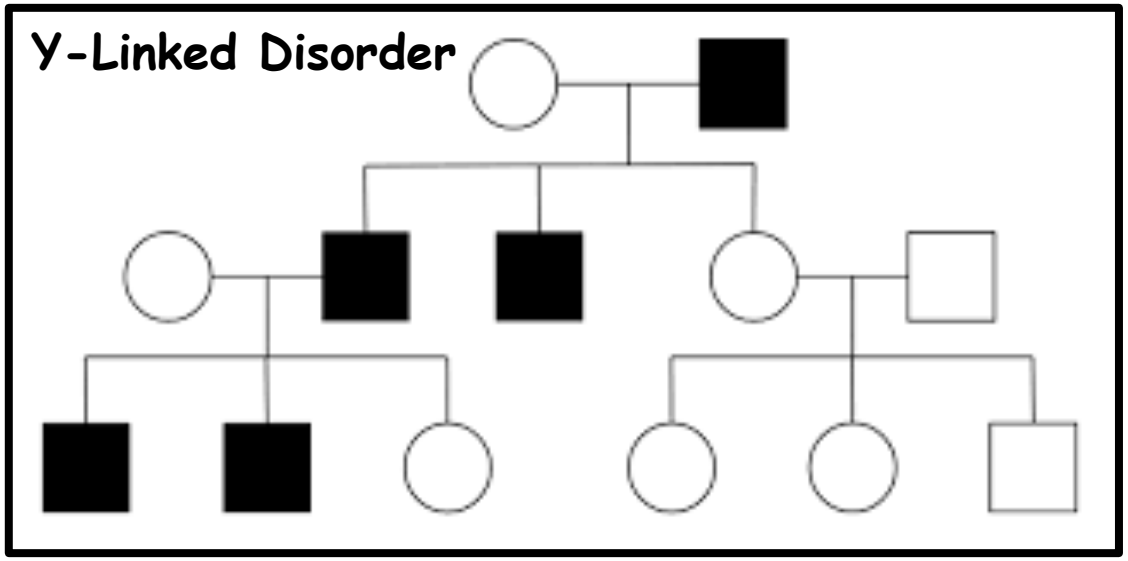

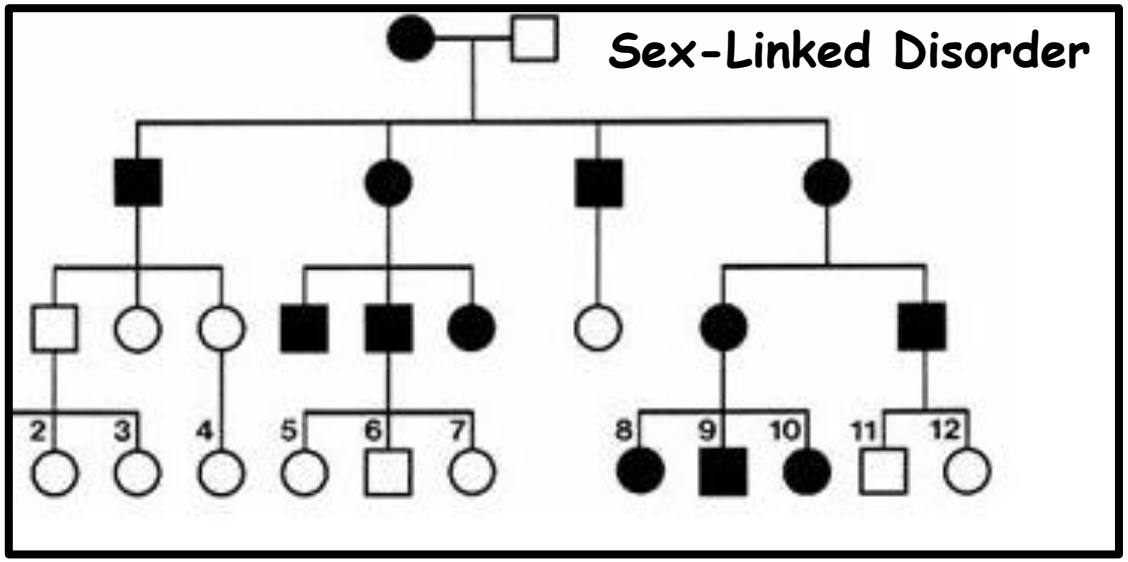

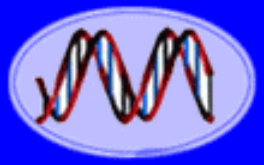

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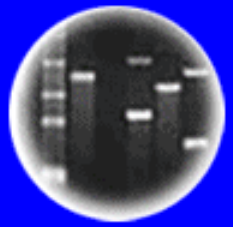

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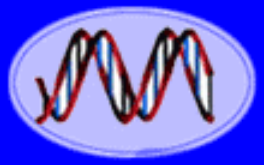
Some Human Genetic Disorders

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	



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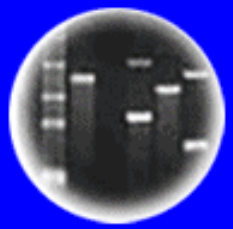

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Cloning: Ethical Issues and Future Consequences

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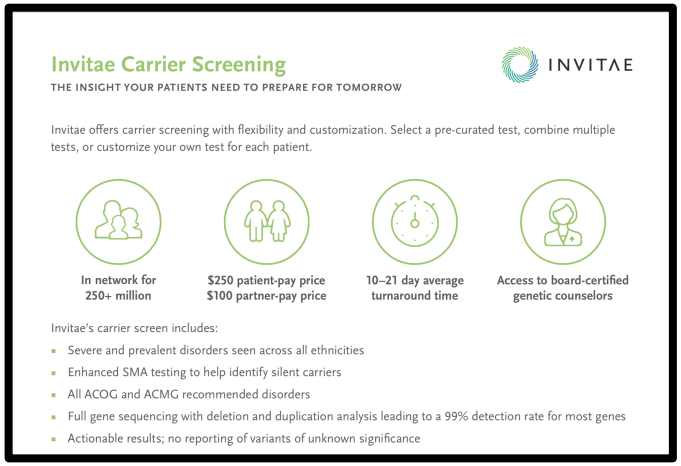
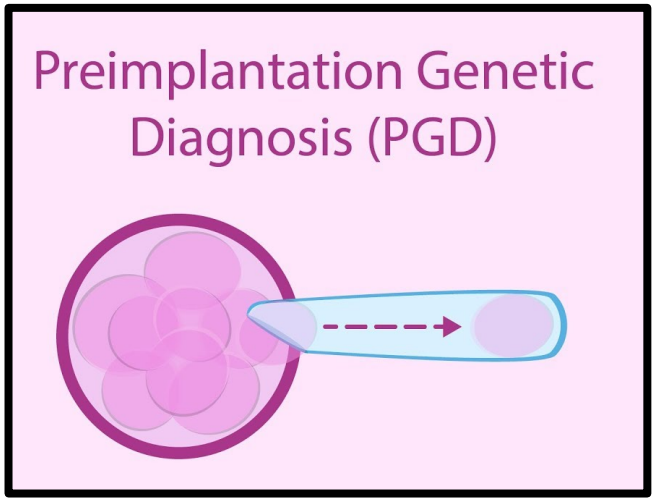
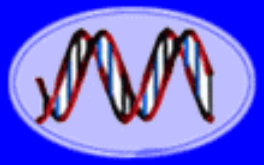
Genetic Testing and Carrier Screening Can Detect Human Genetic Disorders in Parents and Children Before and After Birth

	INVITAE CORE CARRIER SCREEN	INVITAE BROAD CARRIER SCREEN	INVITAE COMPREHENSIVE CARRIER SCREEN
Number of genes	3	46	288
Includes all ACOG & ACMG recommended disorders		●	●
Number of X-linked disorders	1*	5*	21*
Sample type	Blood or saliva	Blood or saliva	Blood or saliva







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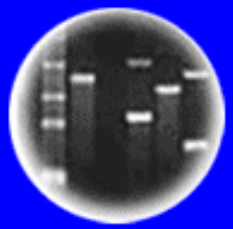

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Cloning: Ethical Issues and Future Consequences

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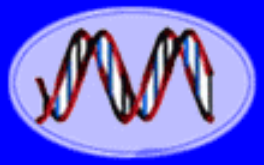
And Newborn Babies Are Screened For Many Genetic Diseases

Category	Condition	Included in California Newborn Screening
Organic Acid Disorders	Propionic Acidemia	✓
	Methylmalonic Acidemia (Methylmalonyl-CoA Mutase)	✓
	Methylmalonic Acidemia (Cobalamin Disorders)	✓
	Isovaleric Acidemia	✓
	3-Methylcrotonyl-CoA Carboxylase Deficiency	✓
	3-Hydroxy-3-Methylglutaric Aciduria	✓
	Holocarboxylase Synthase Deficiency	✓
	β-Ketothiolase Deficiency	✓
	Glutaric Acidemia Type I	✓
Fatty Acid Oxidation Disorders	Carnitine Uptake Defect	✓
	Medium-chain Acyl-CoA Dehydrogenase Deficiency	✓
	Very Long-chain Acyl-CoA Dehydrogenase Deficiency	✓
	Long-chain L-3-Hydroxyacyl-CoA Dehydrogenase Deficiency	✓
	Trifunctional Protein Deficiency	✓
Amino Acid Disorders	Argininosuccinic Aciduria	✓
	Citrullinemia Type I	✓
	Maple Syrup Urine Disease	✓
	Homocystinuria	✓
	Classic Phenylketonuria	✓
	Tyrosinemia Type I	✓
Endocrine Disorders	Primary Congenital Hypothyroidism	✓
	Congenital Adrenal Hyperplasia	✓
Hemoglobin Disorders	S,S Disease (Sickle Cell Anemia)	✓
	S, β-Thalassemia	✓
	S,C Disease	✓
Other Disorders	Biotinidase Deficiency	✓
	Cystic Fibrosis[3]	✓
	Classic Galactosemia	✓
	Glycogen Storage Disease Type II (Pompe)	✓
	Mucopolysaccharidosis Type I	✓
	Severe Combined Immunodeficiencies	✓
	X-linked Adrenoleukodystrophy	✓
	Critical Congenital Heart Disease	✸
	Hearing Loss	✸
	Spinal Muscular Atrophy	Planning for 2020

BabyGenes™



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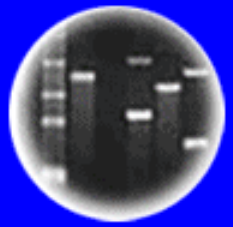

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Cloning: Ethical Issues and Future Consequences

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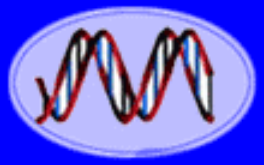
Treatments Have Been Developed For Genetic Diseases We Have Discussed in HC70A

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



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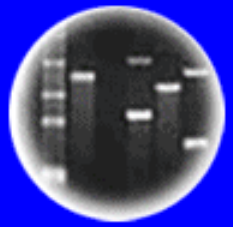

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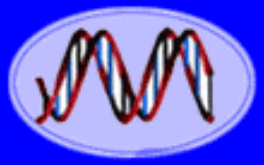
What Are the Prospects For a Permanent "Cure" For Genetic Diseases Using Gene Therapy?



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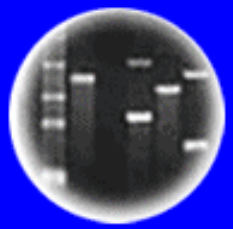

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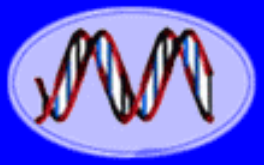
What Information is Needed Before Initiating Development of a Gene Therapy?

1. What Is Known About the Biology of the Disorder?
2. Does the Condition Result From a Mutation in One or More Genes?
3. Is the Mutant Gene Dominant or Recessive?
4. Has the Affected Gene Been Isolated?
5. Will Adding a Normal Copy of the Gene Fix the Problem in the Affected Tissue?
6. Can You Deliver the Gene to Cells of the Affected Tissue?
7. What Vector or Approach Should be Used?



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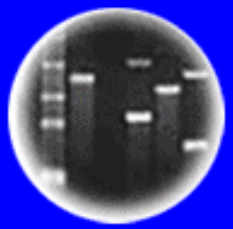

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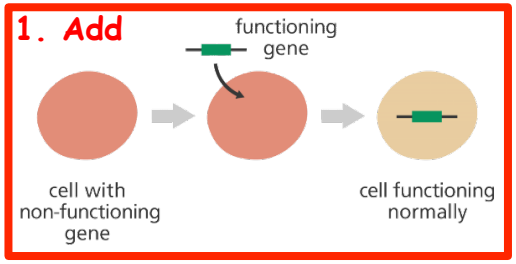
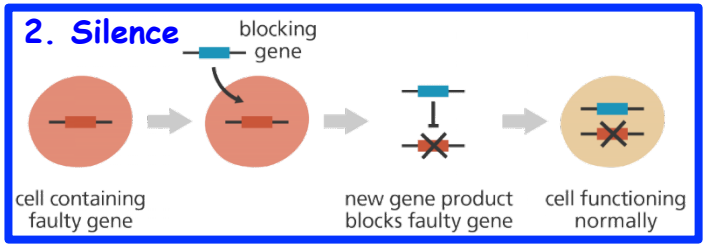
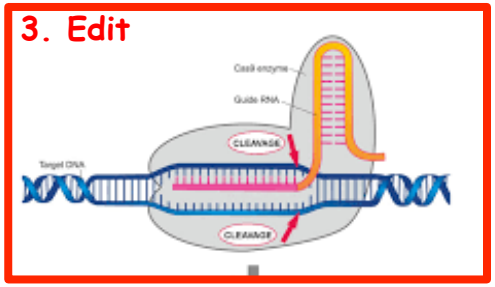
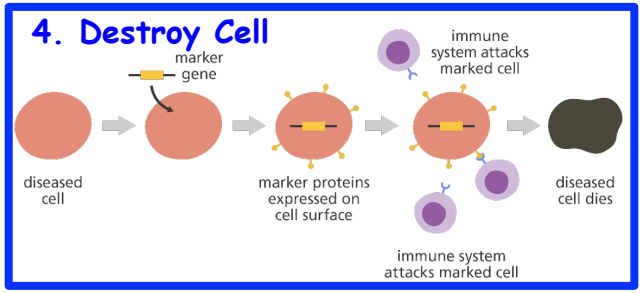
Gene Therapy Strategies

1. Gene Addition
   a. Recessive Genetic Diseases
2. Gene Silencing
   a. Dominant Genetic Diseases
3. Gene Editing
   a. Dominant & Recessive Genetic Diseases
   b. Silence or Correct Mutant Genes
4. Targeted Destruction of Specific Cell-Types
   a. Engineer Cells With "Killer Gene"



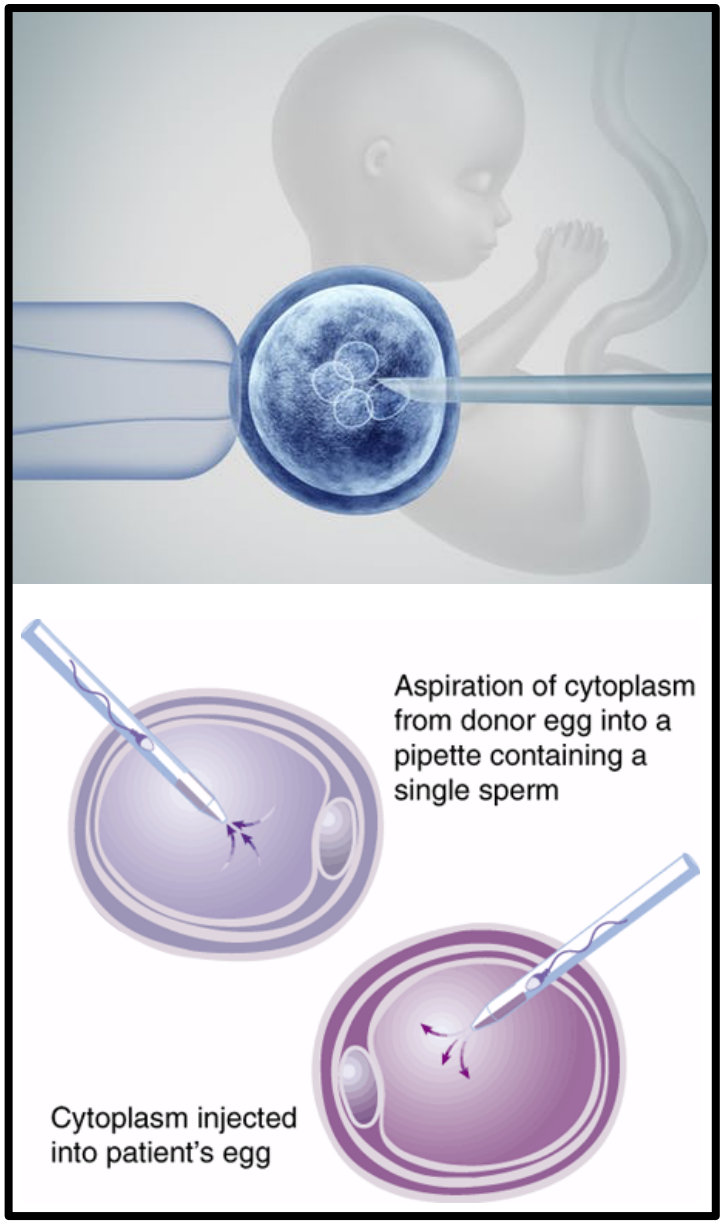


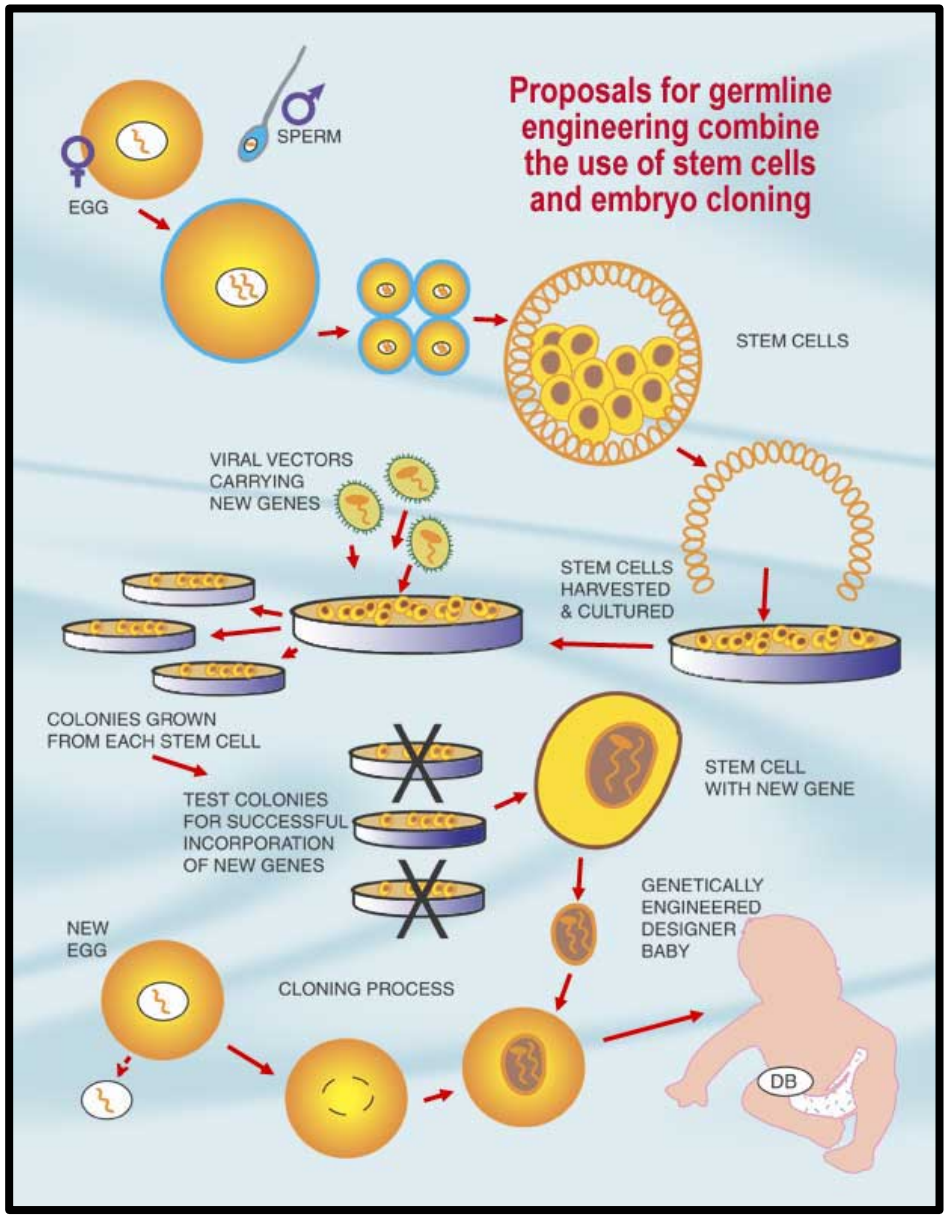




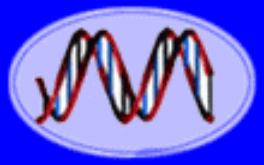
Humans Have Been Genetically Engineered to Cure Genetic Diseases (Human GMOs!!)

Germline Gene Therapy





Passed on to Future Generations



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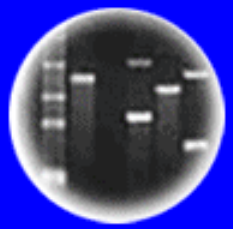
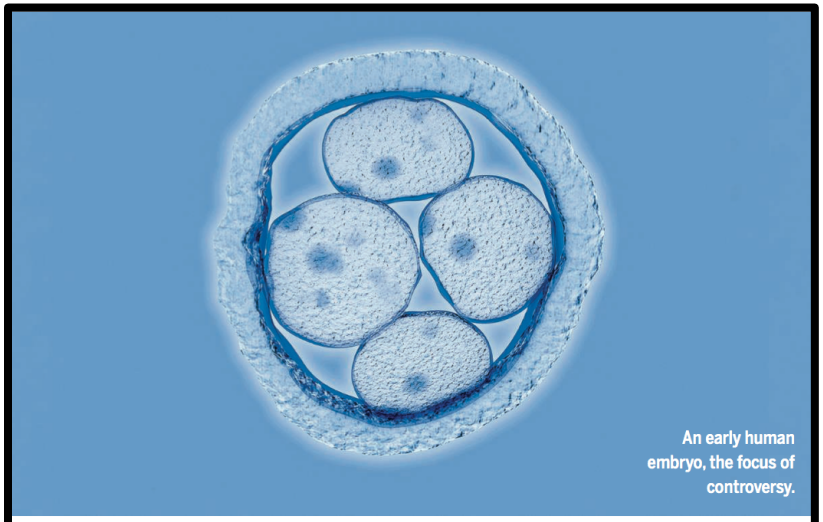


DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow



An early human embryo, the focus of controversy.

BIOETHICS

Embryo engineering alarm

Researchers call for restraint in genome editing

Germline Gene Therapy Has Been Used in Humans!!

Genome-edited baby claim provokes international outcry

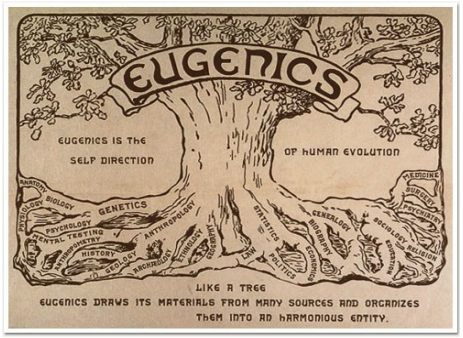
Scientists Seek Ban on Method of Editing the Human Genome

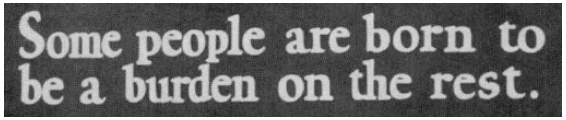
By NICHOLAS WADE MARCH 19, 2015

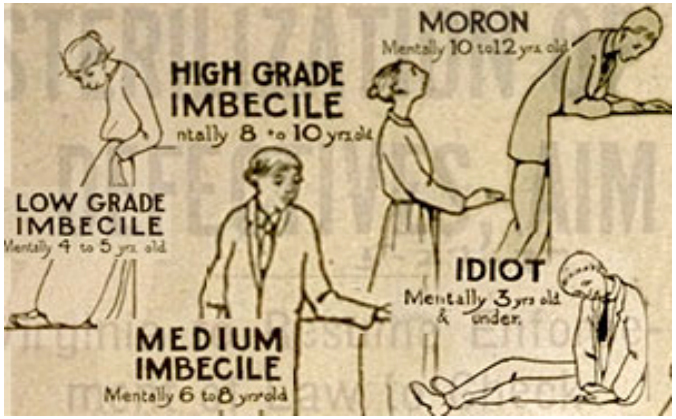
A group of leading biologists on Thursday called for a worldwide moratorium on use of a new genome-editing technique that would alter human DNA in a way that can be inherited.

Chinese Scientist Who Genetically Edited Babies Gets 3 Years in Prison

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







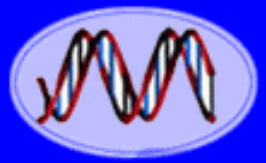

DECISION HELD
STEP TOWARD
A SUPER-RACE

Health Official Praise Court in Upholding Sterilization Law

WASHINGTON, May 6.—(INS)—An improved race of Americans is on its way as a result of the Supreme

- 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?

HC70A Examples of Animal & Plant Germline Gene Therapy



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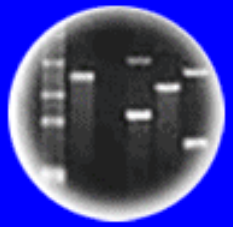

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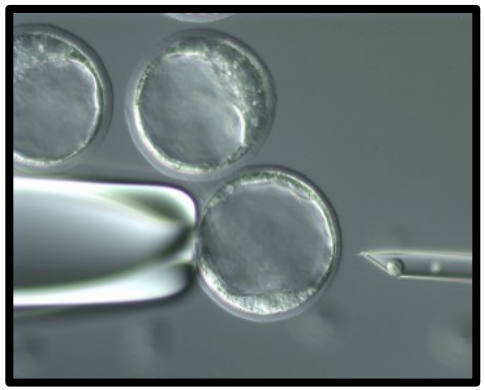
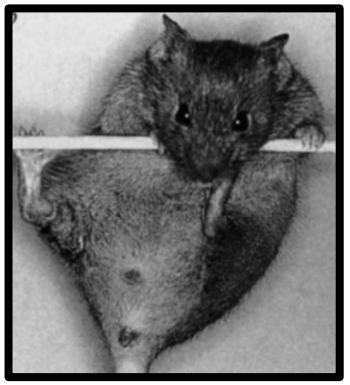
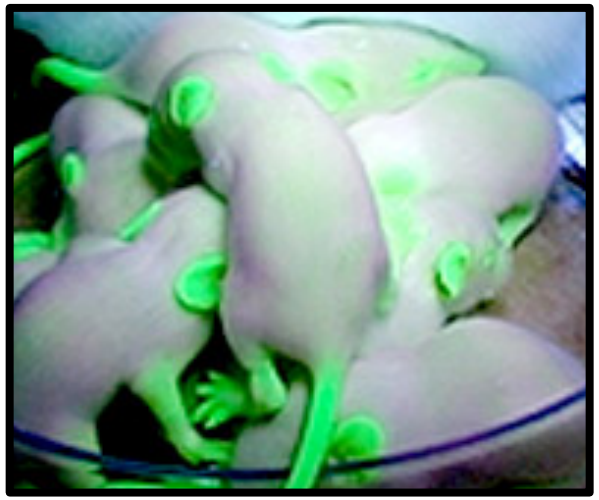
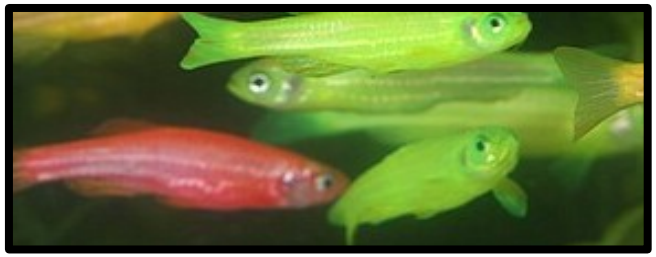
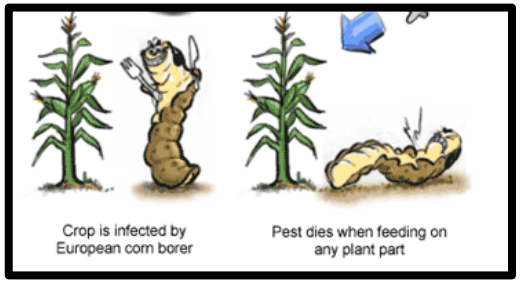


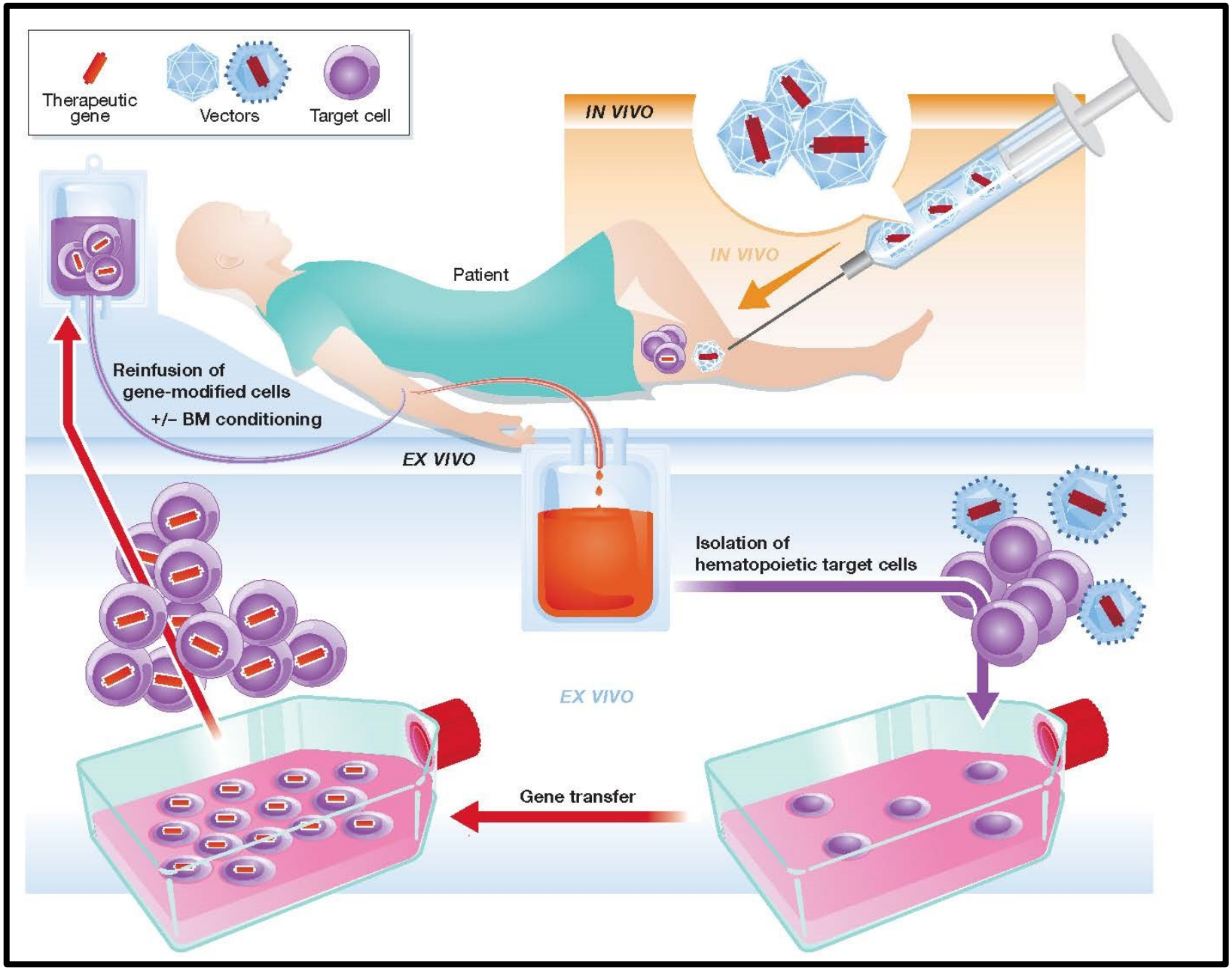






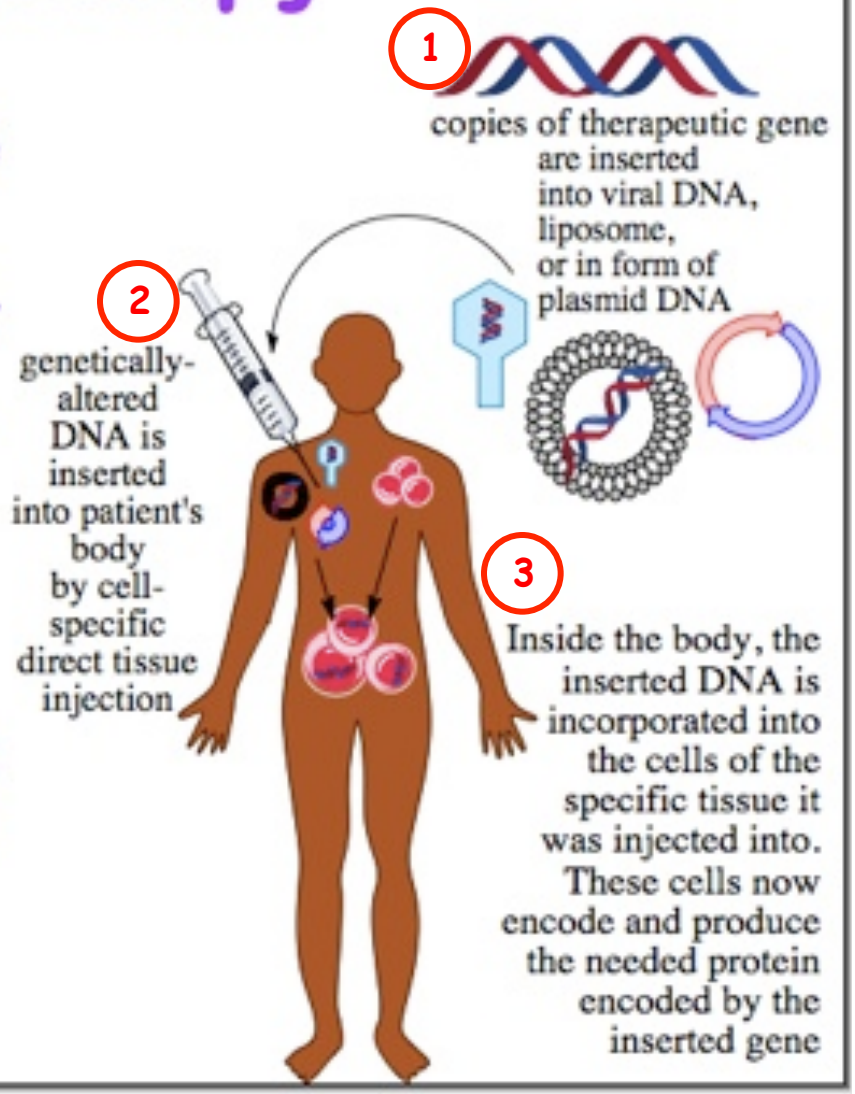


Somatic Cell Gene Therapy – *In Vivo* and *Ex Vivo*



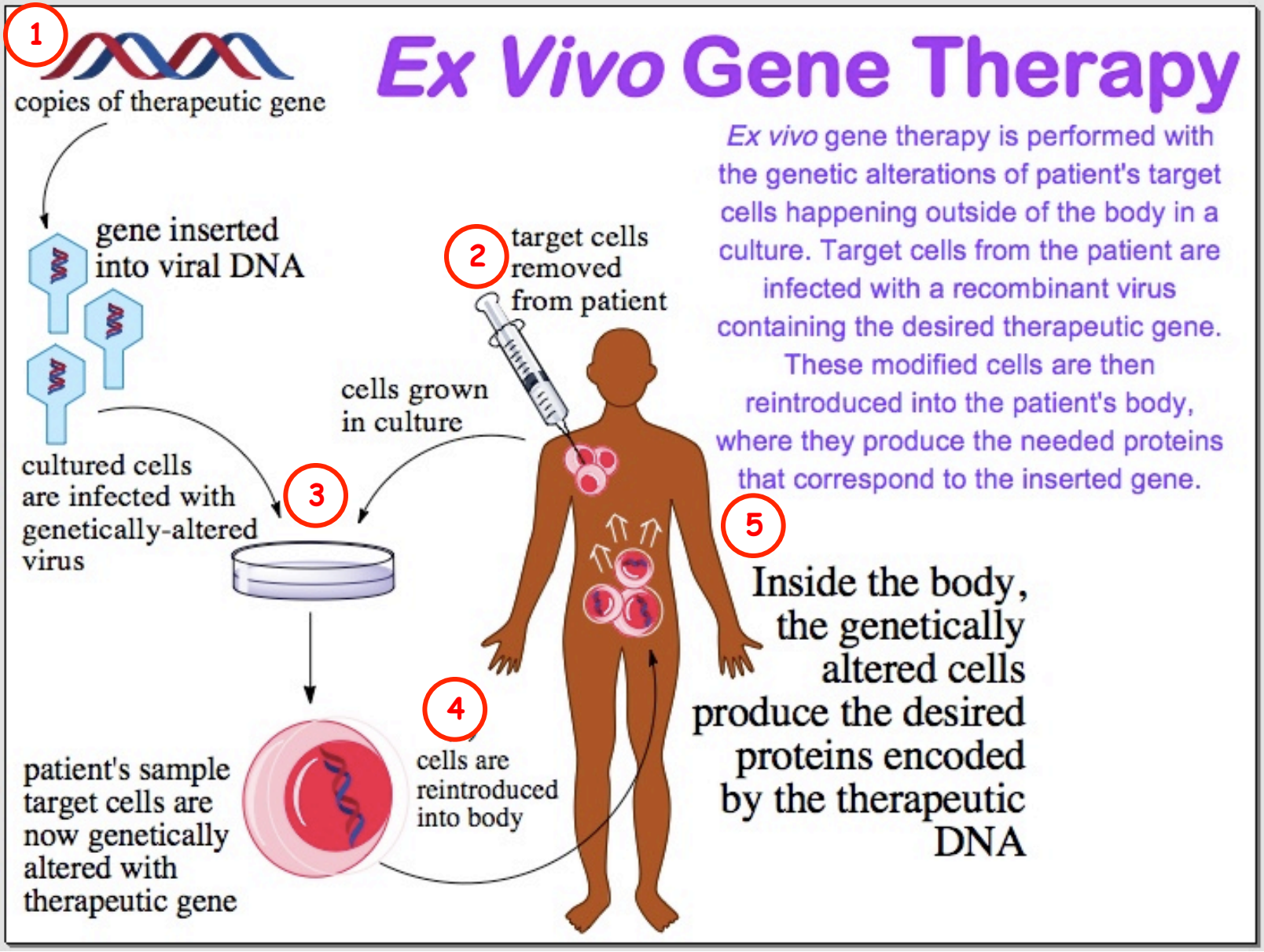
In Vivo Gene Therapy

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.



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.

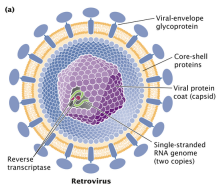


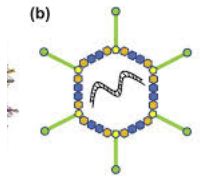
Viral Vectors Used to Deliver Genes to Cells in Somatic Cell Gene Therapy

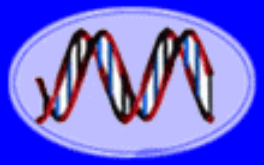
Table 1 Viral vectors discussed in this review

Features	Retroviral	Lentiviral	Adenoviral	AAV
Viral genome	RNA	RNA	DNA	DNA
Cell division requirement for target cell	Yes	G1 phase	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

Natural Process – High Efficiency
Similar to Bacteriophages and Agrobacterium







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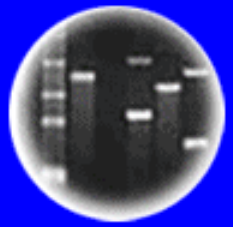
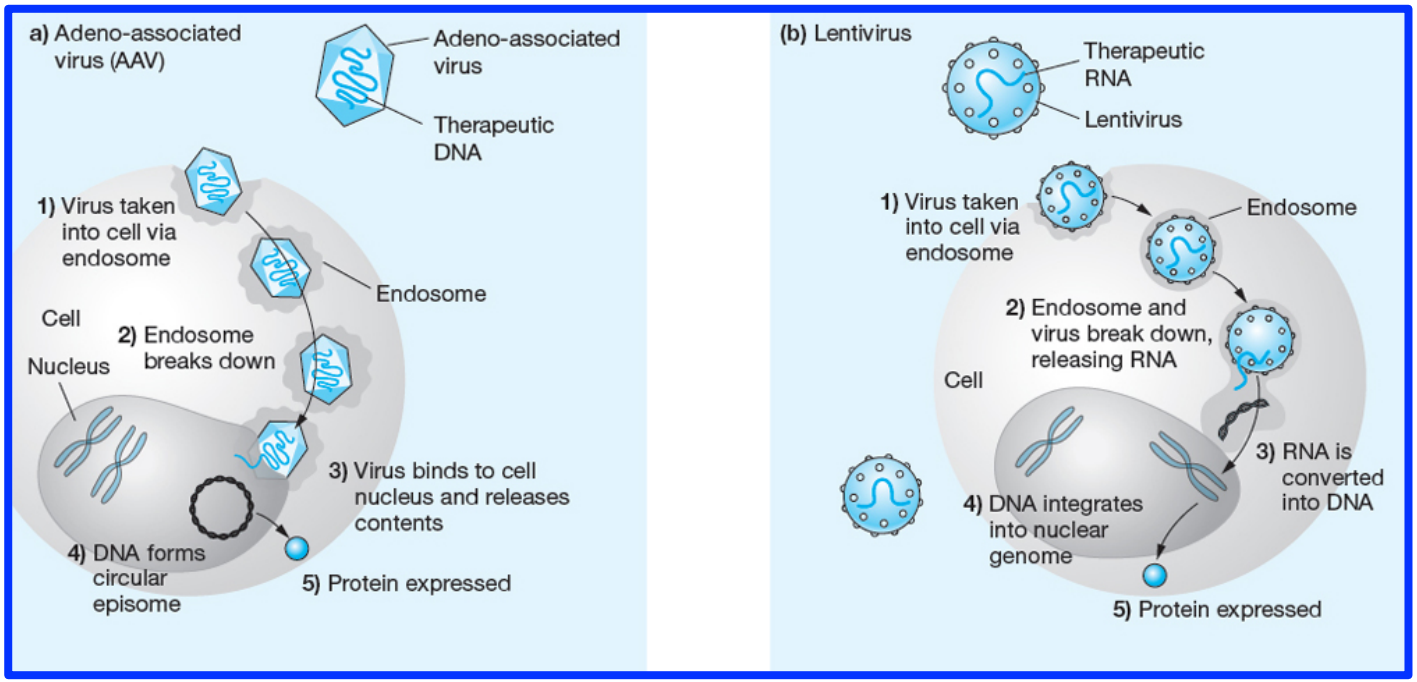


DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

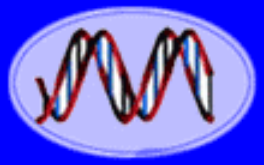
Plants of Tomorrow

Retrovirus and Adeno-associated Virus Life Cycles



In Vivo Gene Therapy

Ex Vivo Cell Gene Therapy



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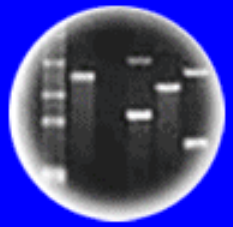

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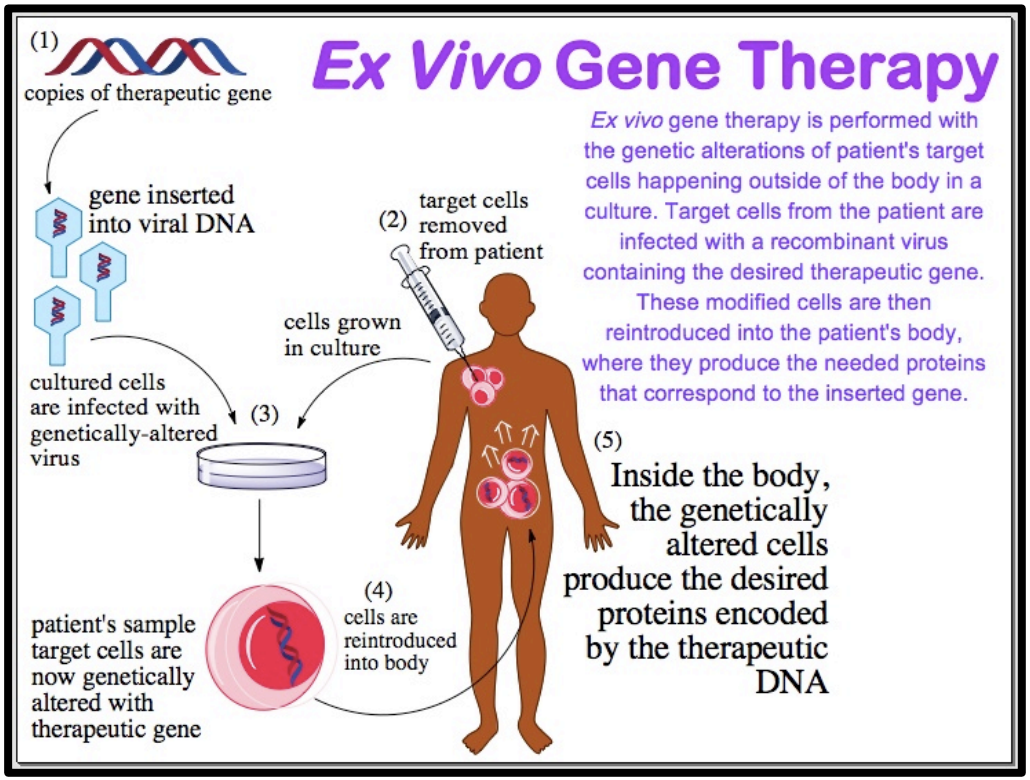
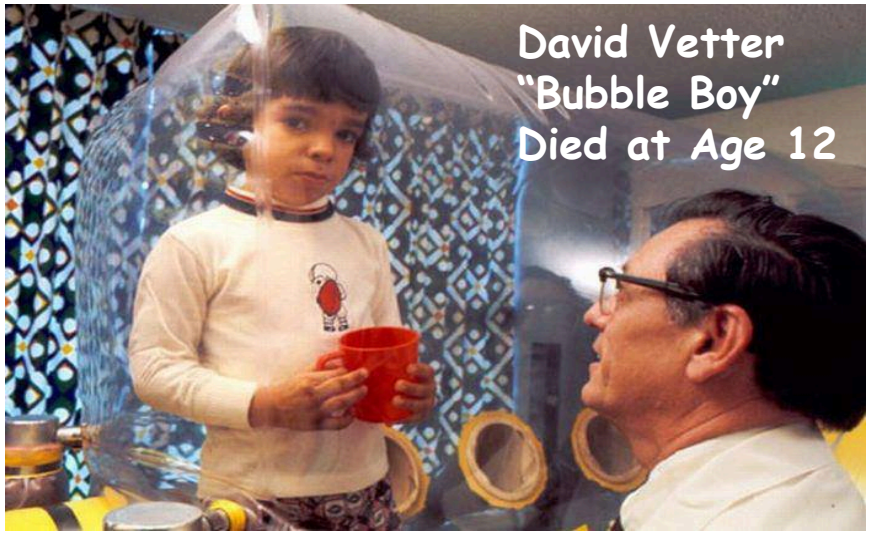
Case Study of Using Retrovirus *Ex Vivo* Gene Therapy for Severe Combined Immunodeficiency (SCID)



Replacement of Recessive Mutant Genes

Severe Combined Immunodeficiency Diseases (SCID)

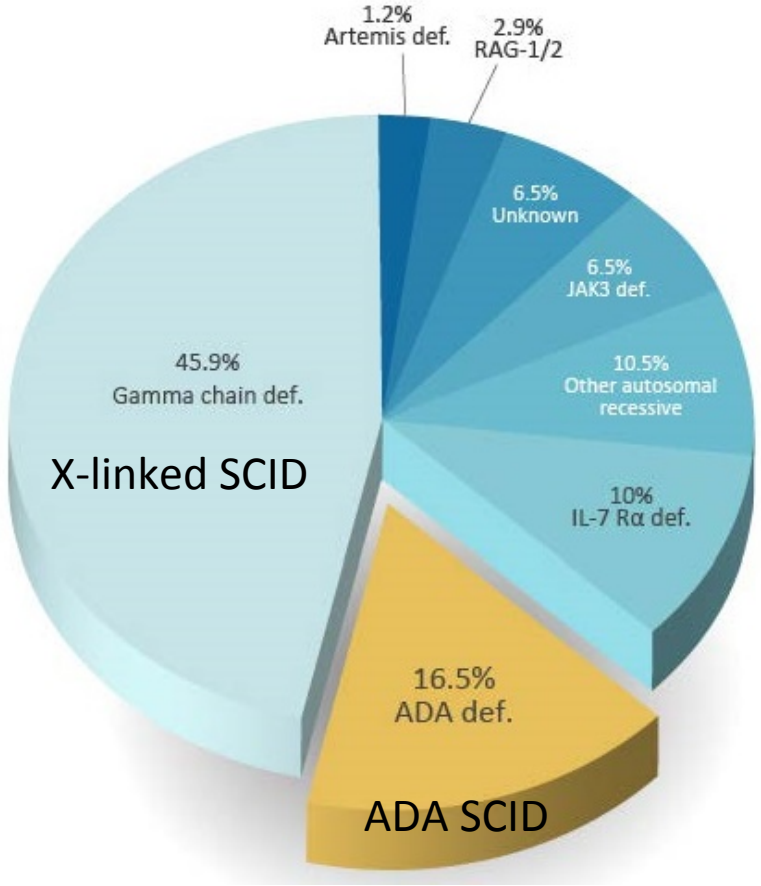


A Group of Rare, Sometimes Fatal, Congenital Disorders Characterized by Little or No Immune Response.

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

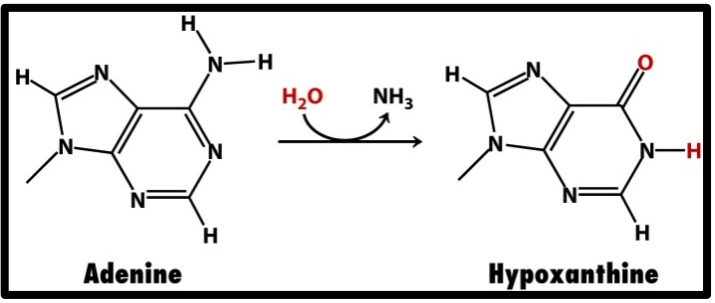
Relative Frequency of the Different Molecular Defects in SCID



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

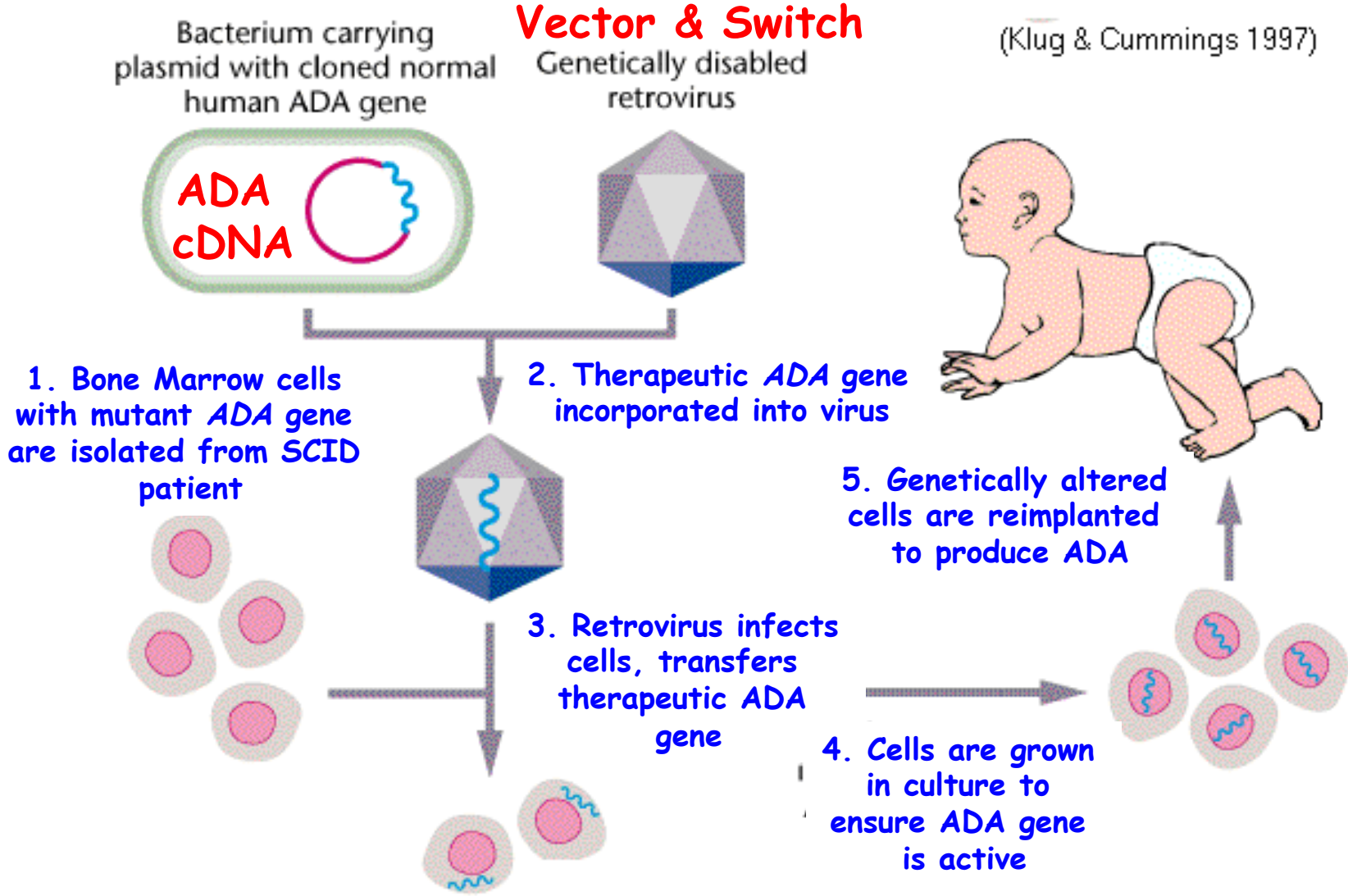


Degradation of Adenosine

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

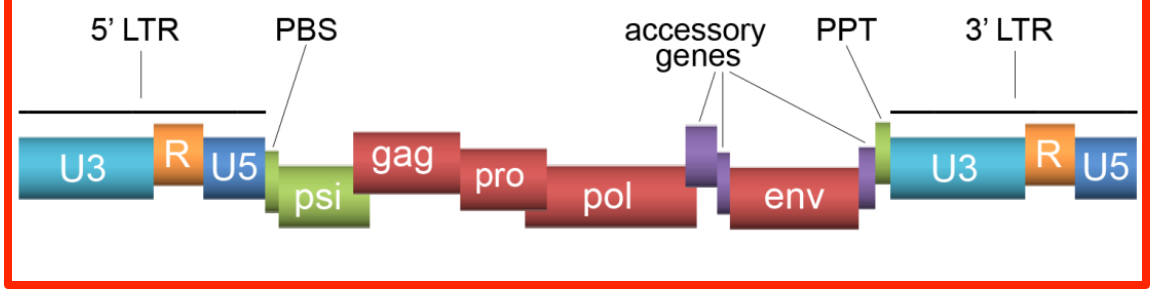
Ex Vivo Gene Therapy for ADA-Severe Combined Immunodeficiency (SCID)



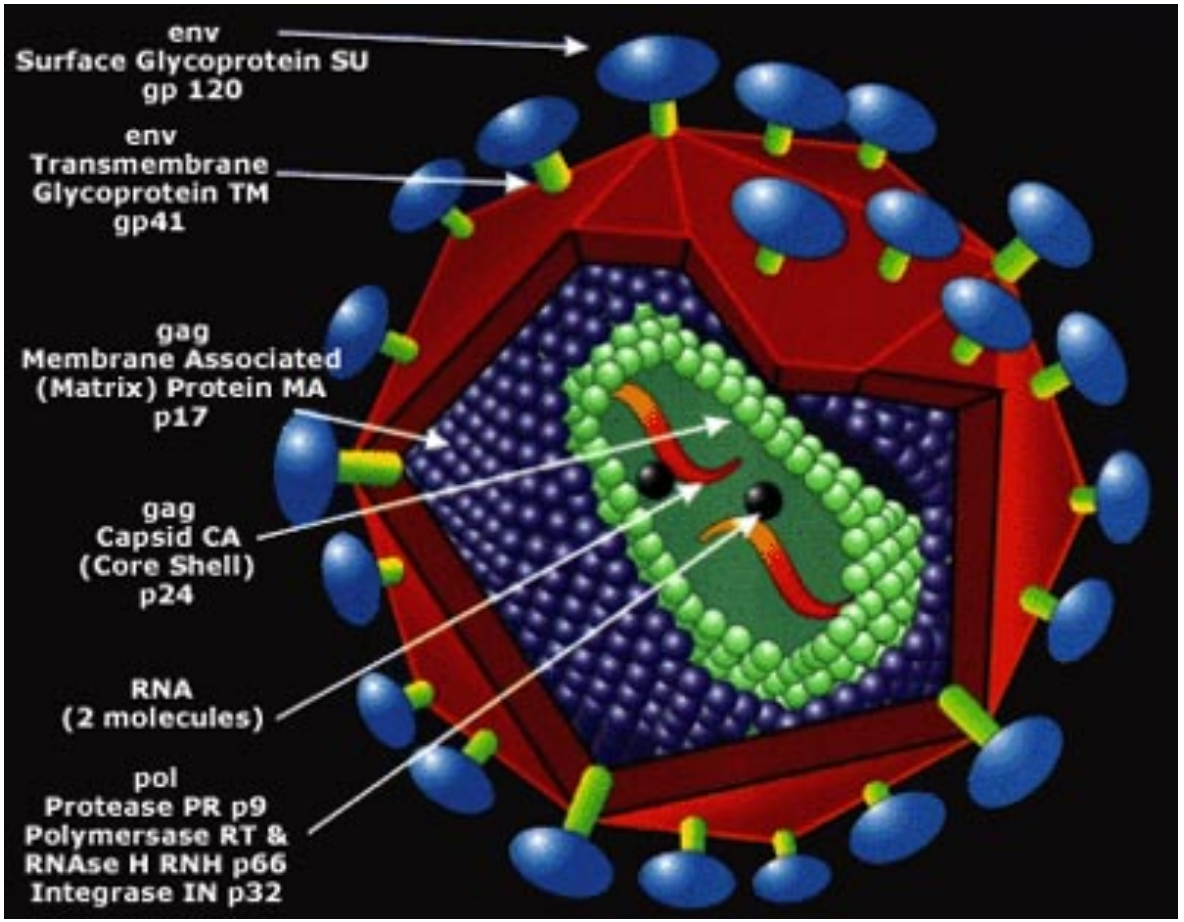
ADA-SCID Clinical Trial Started in 1990

A Retrovirus and Its Genome

7-12 kb RNA Genome

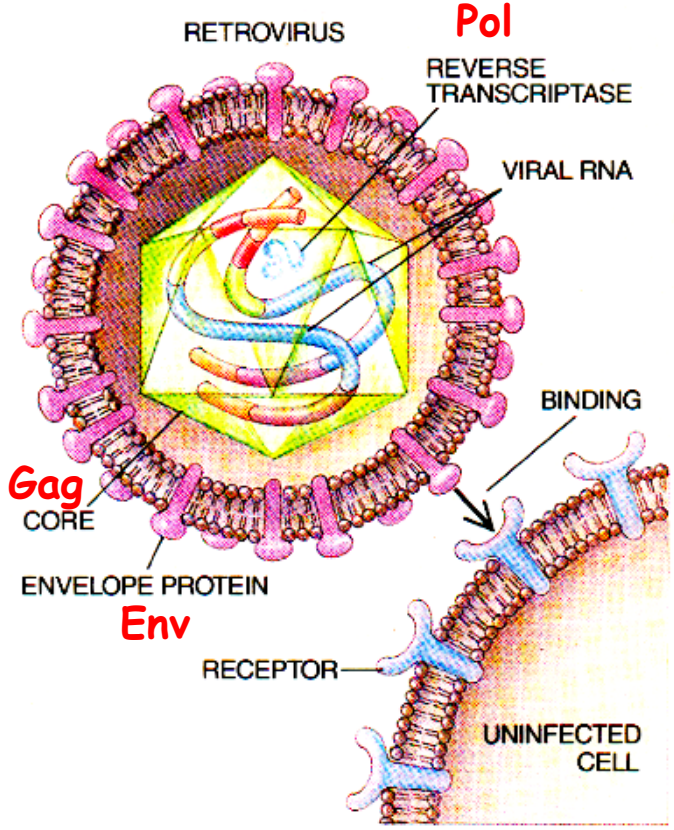


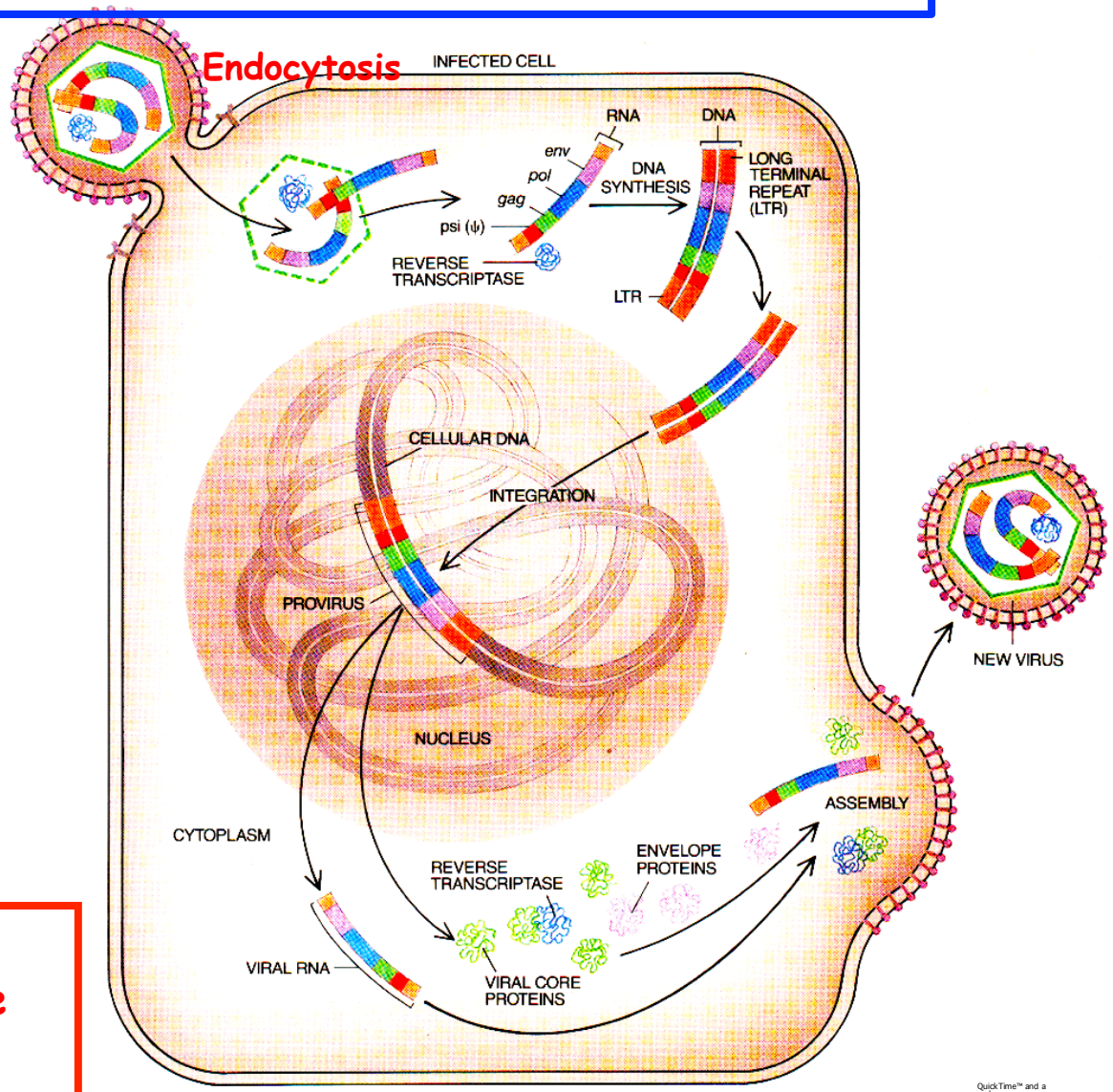
- 5' long terminal repeat (LTR) – strong switch & integration
- 3' LTR – strong switch, integration & transcriptional termination
- psi (Ψ) – packaging element needed to package the RNA genome into the viral particle
- gag – structural (coat) proteins
- pro - protease
- pol – reverse transcriptase
-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



Retrovirus Life Cycle

Retroviruses Integrate Their Genomes Into Human Cells

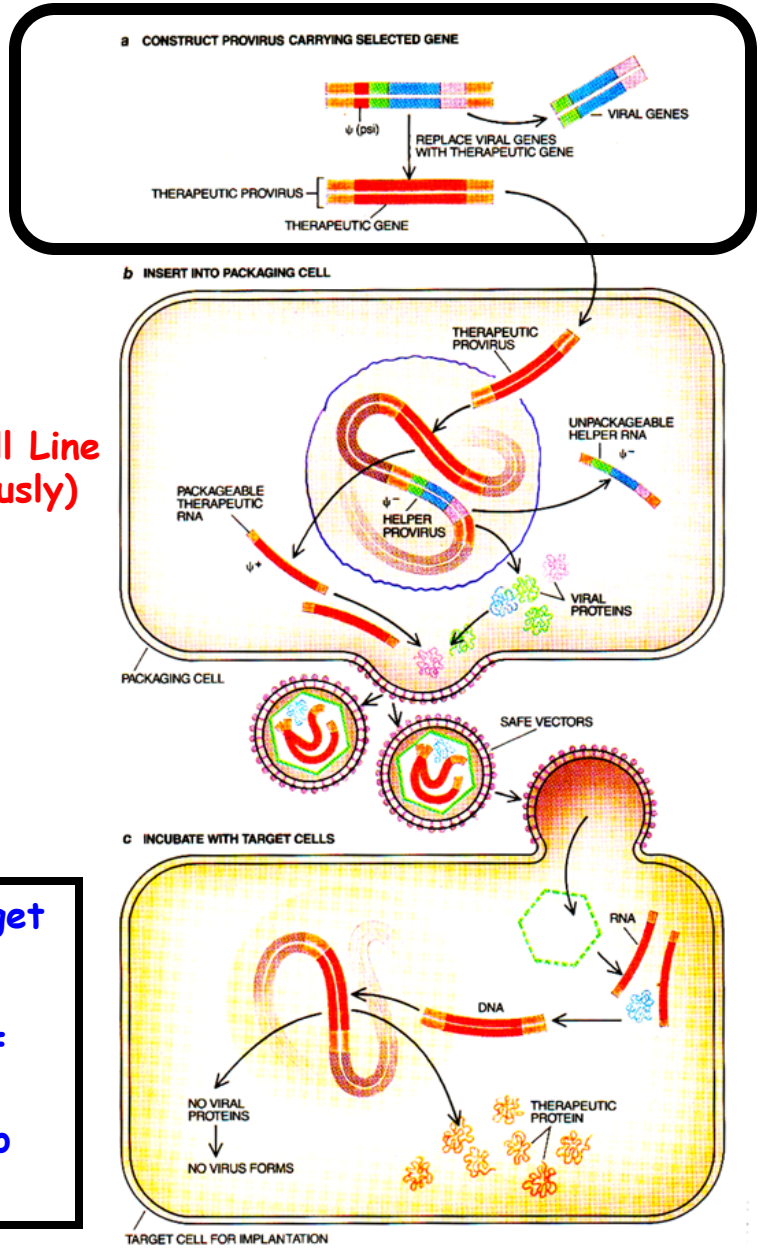




- Gag = Capid Protein
- Pol = Reverse Transcriptase
- Env = Envelope Protein
- Ψ (Psi) = Packaging Sequence
- LTR = Strong Switch

QuickTime™ and a decompressor are needed to see this picture.

Using Retroviruses for Ex Vivo Gene Therapy



A.

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

B.

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

Packaging Cell Line (Made Previously)

C.

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

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

Did the Gene Therapy Strategy Work?

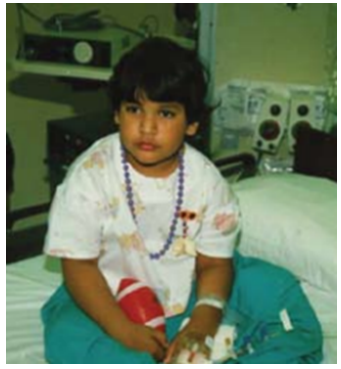
Science
AAAS

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

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

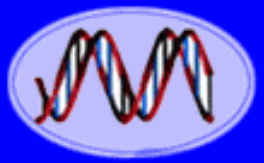
In 1990, a clinical trial was started using retroviral-mediated transfer of the adenosine deaminase (ADA) gene into the T cells of two children with severe combined immunodeficiency (ADA⁻ SCID). The number of blood T cells normalized as did many cellular and humoral immune responses. Gene treatment ended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

- ADA Gene Expression In T Cells Persisted After Four Years
- But - Patients Remained On ADA Enzyme Replacement Therapy Throughout The Gene Therapy Treatment



Ashanthi DeSilva
1992

Ashanthi DeSilva
2020



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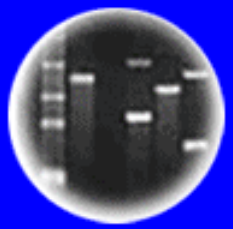

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Some Early Problems with Human Gene Therapy

- Inefficient Delivery Of Vector To Target Cells
- Low Expression Level Of Therapeutic Gene
- Adverse Immune Reactions To Vector
- 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)

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

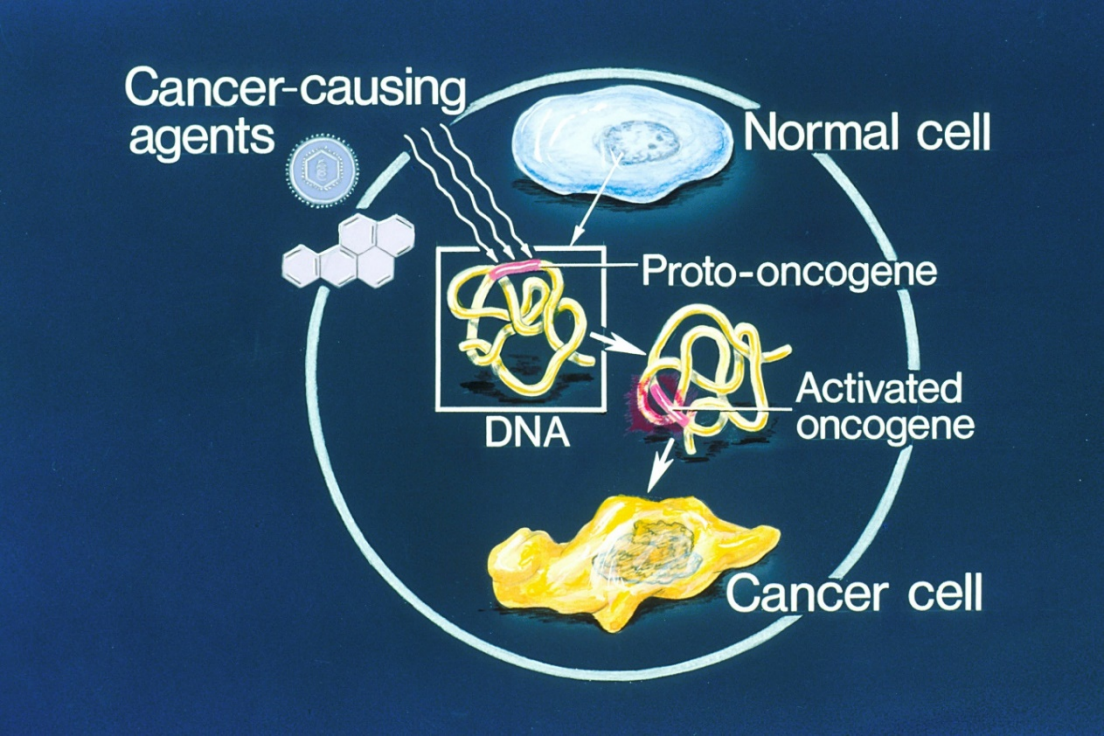
TRIALS ARE HALTED ON A GENE THERAPY

By SHERYL GAY STOLBERG
Published: October 4, 2002

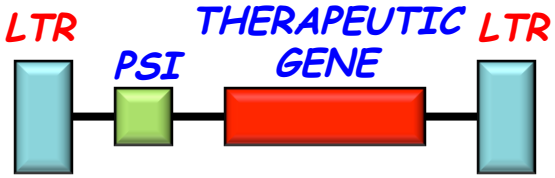
WASHINGTON, Oct. 3— Officials in the United States and France said today that they had suspended four gene therapy experiments because the treatment, which cured a 3-year-old boy of a fatal immune deficiency, may have given him an illness similar to leukemia.

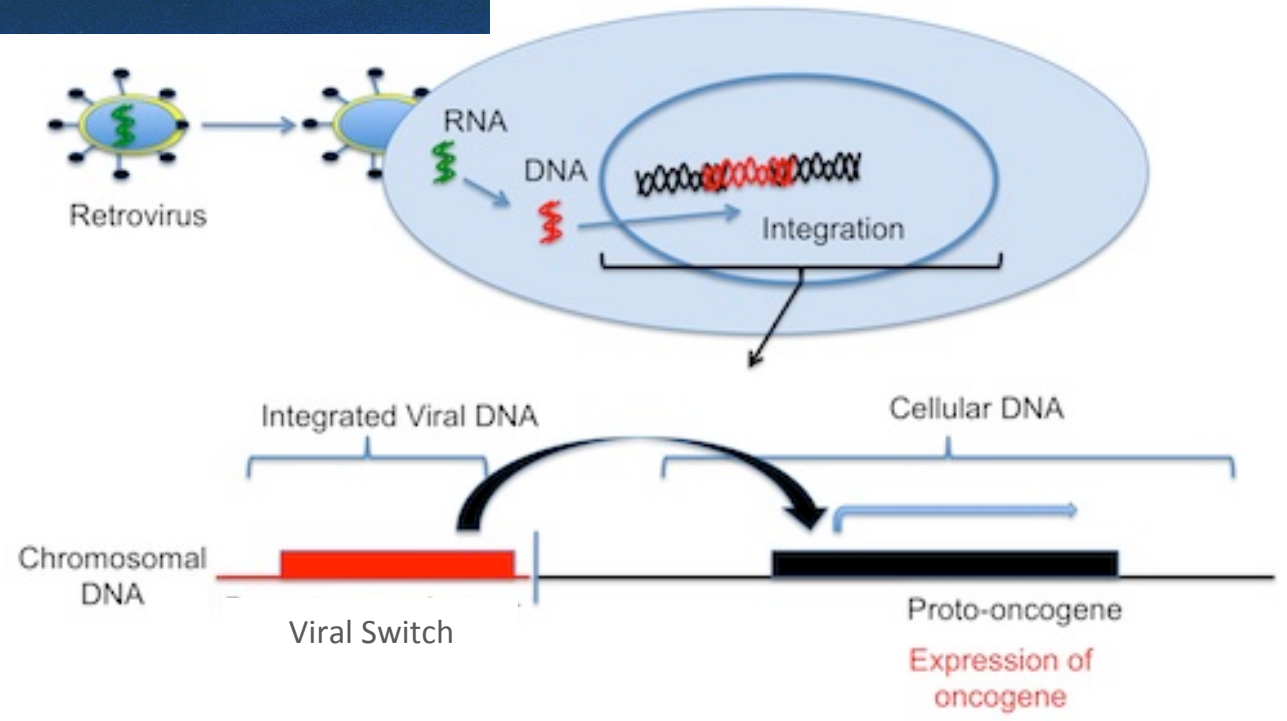
- 3 Of 17 Patients In Clinical Trial For X-SCID Gene Therapy Developed Clonal Lymphoproliferative Disorder – A Leukemia
- The Leukemia Was Caused By Insertion Of Retrovirus Near Proto-oncogenes And Activation Of These Proto-oncogenes By Retroviral Switches

Retroviral Switches Can Activate Proto-Oncogenes and Induce Cancers

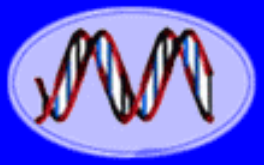


A Typical Retrovirus Gene Therapy Vector





Viral Switch



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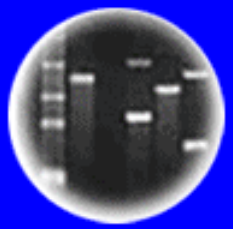


DNA Fingerprinting

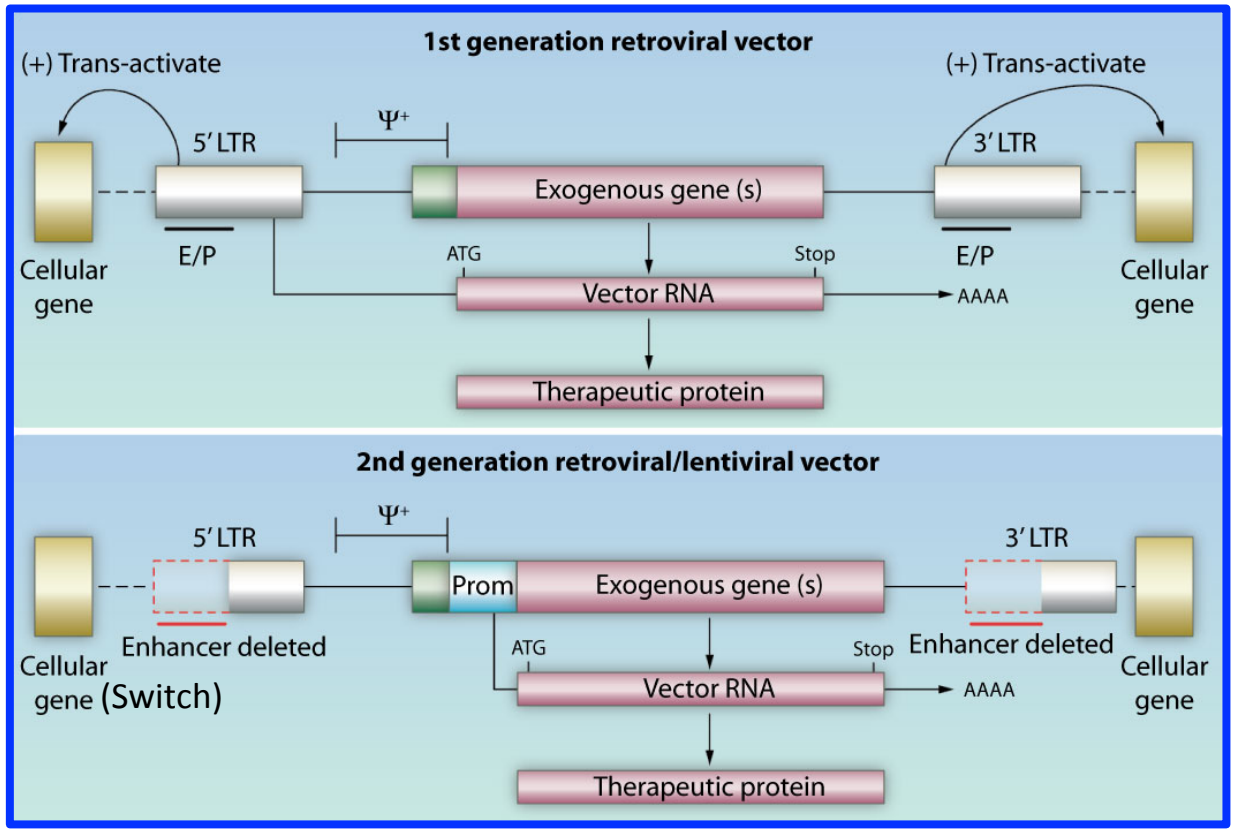
Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

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
- Develop New And Safer Vectors
  - Lentivirus
  - Adeno Associated Virus

Development of Self-Inactivating (SIN) Lentiviral 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.

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

Strimvelis®

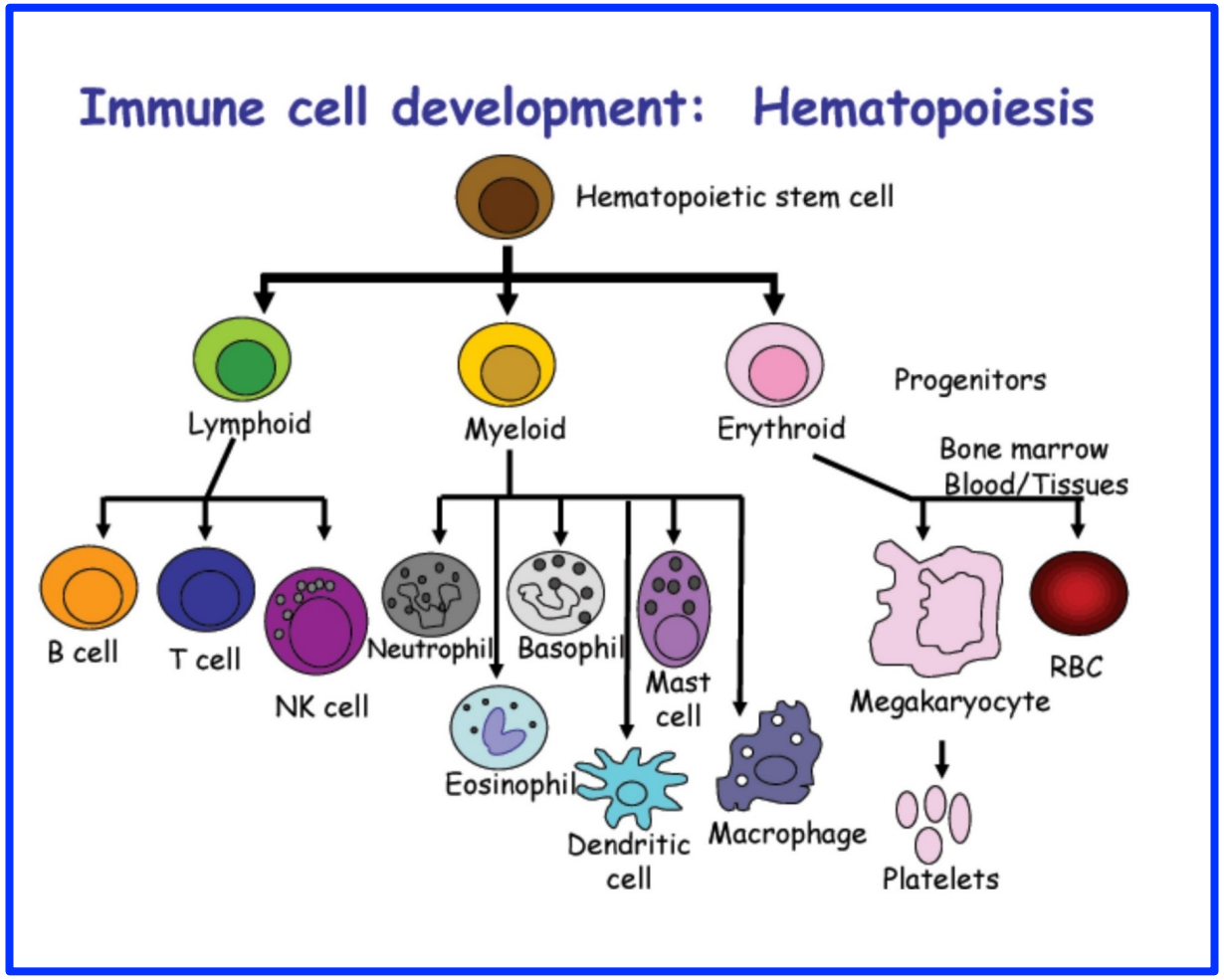
EMA APPROVED

Results After 10 Years

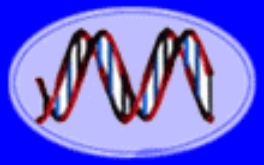
- ADA-SCID – 4 of 6 Children Experienced Immune Reconstitution
- SCID-X1 – 9 of 10 Children Experienced Normal T-cell Number
- But - 5 of 20 SCID-X1 Subjects Experienced Leukemia-like T Lymphoproliferation In Another Study

- 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

Ex Vivo Retrovirus Gene Therapy Combined with Blood Stem Cells Can Target Other Blood Diseases



- SCID Artemis
- SCID Rag-1
- SCID ADA
- Sickle Cell Disease
- Thalassaemia
- Chronic Granulomatous Disease
- Leucocyte Adhesion Deficiency
- Wiskott Aldrich Syndrome
- X-linked Lymphoproliferative Syndrome



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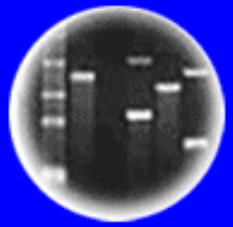

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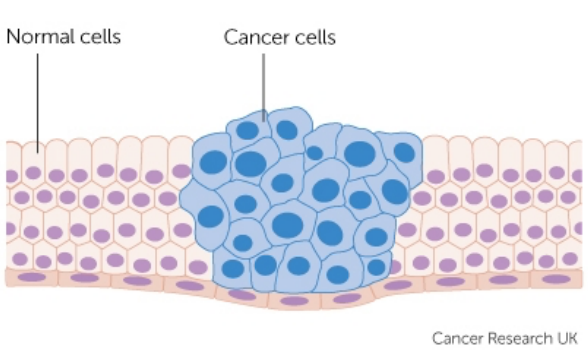
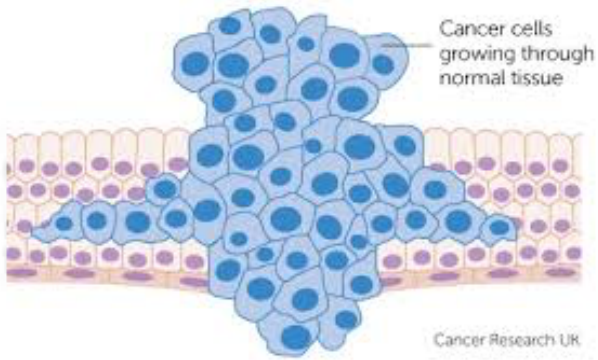
Using *Ex Vivo* Gene Therapy to Cure Cancer

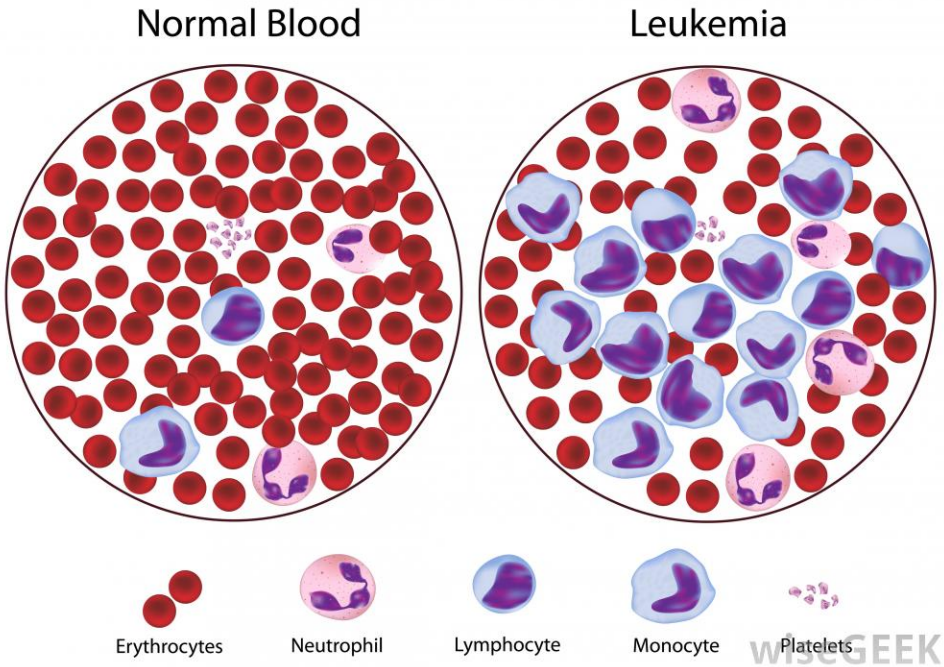
Cell Engineering





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

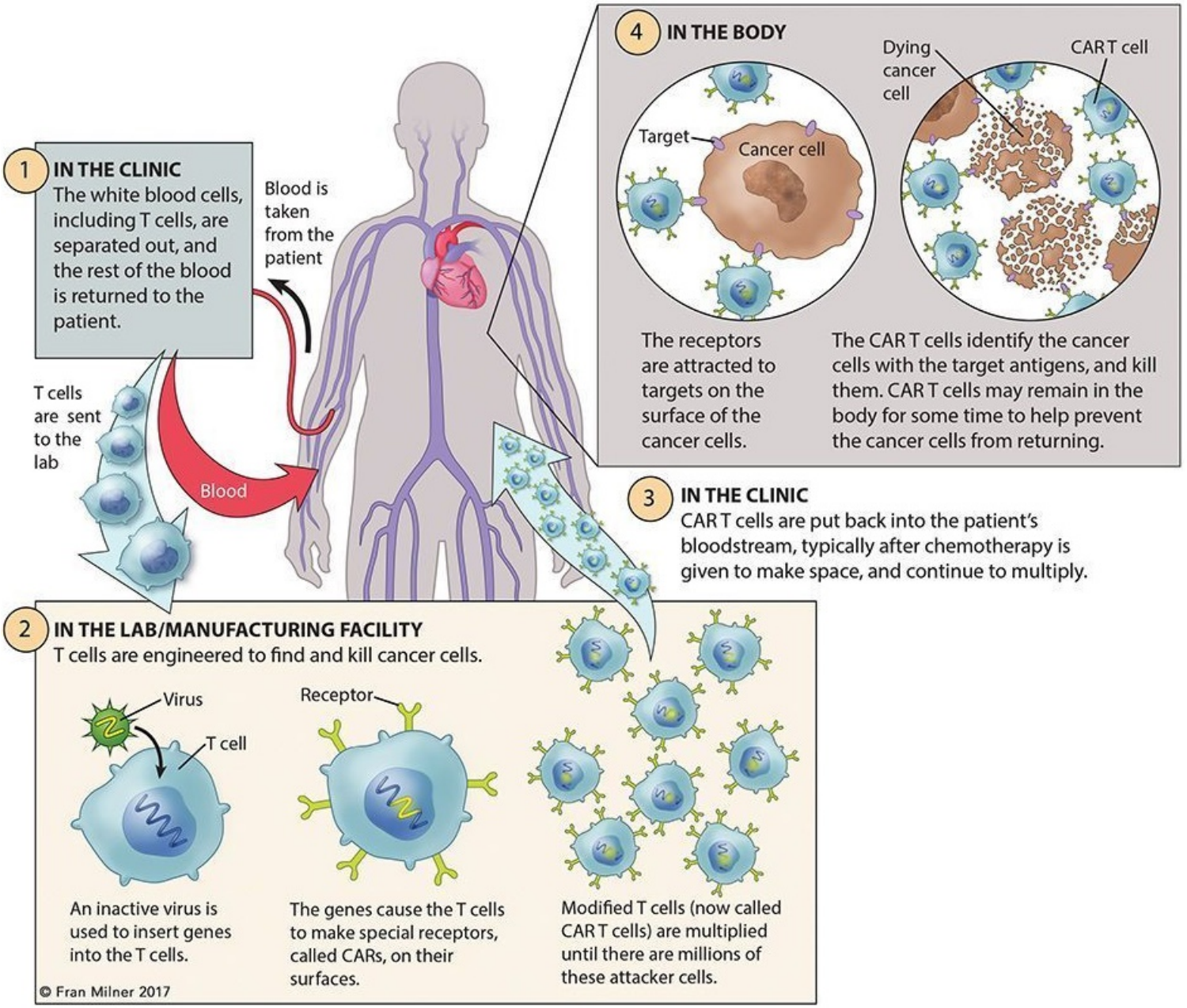
Leukemia Is Cancer of the Blood, That Results in an Increase in Immature White Blood Cells. Chronic Lymphoid Leukemia Affects B Cell Lymphocytes



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

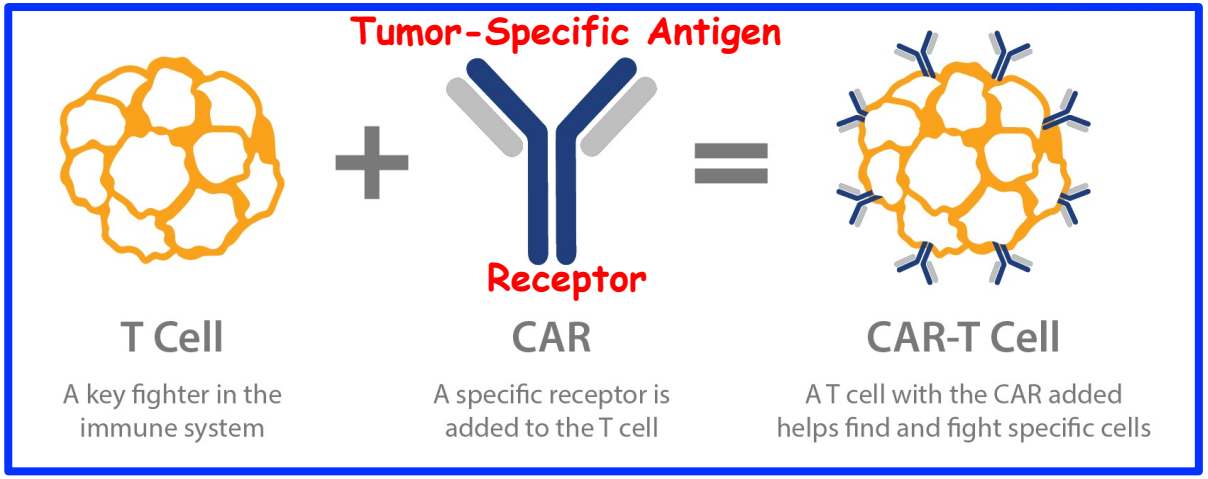
Chimeric Antigen Receptor (CAR-T) Cell Strategy

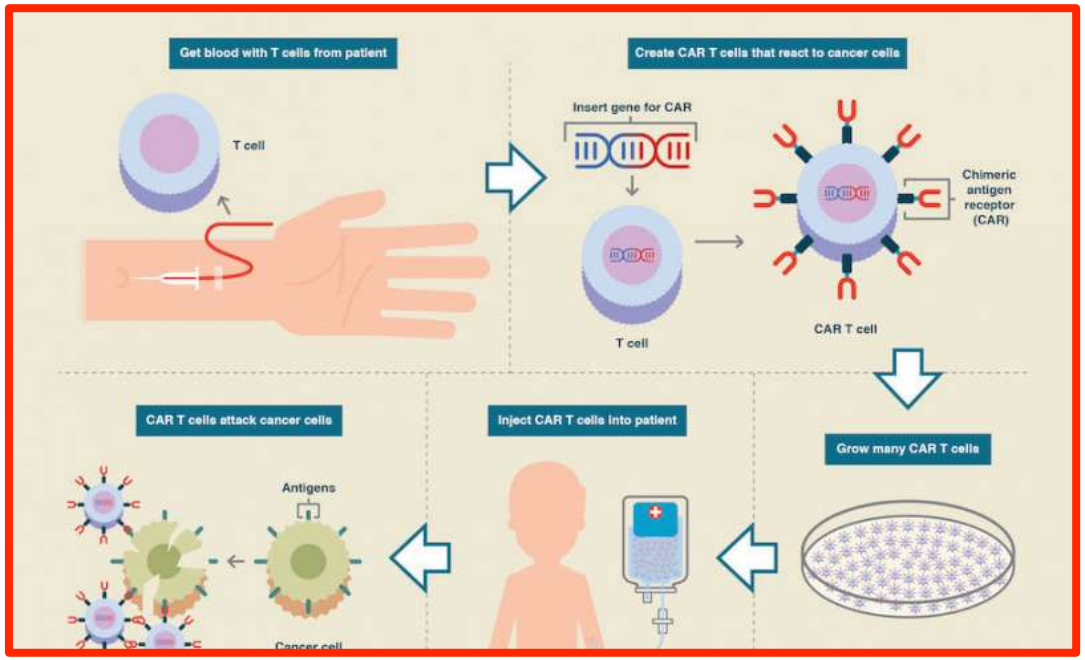


Ex-Vivo CAR-T Gene Therapy for Chromic Lymphocytic Leukemia (CLL)

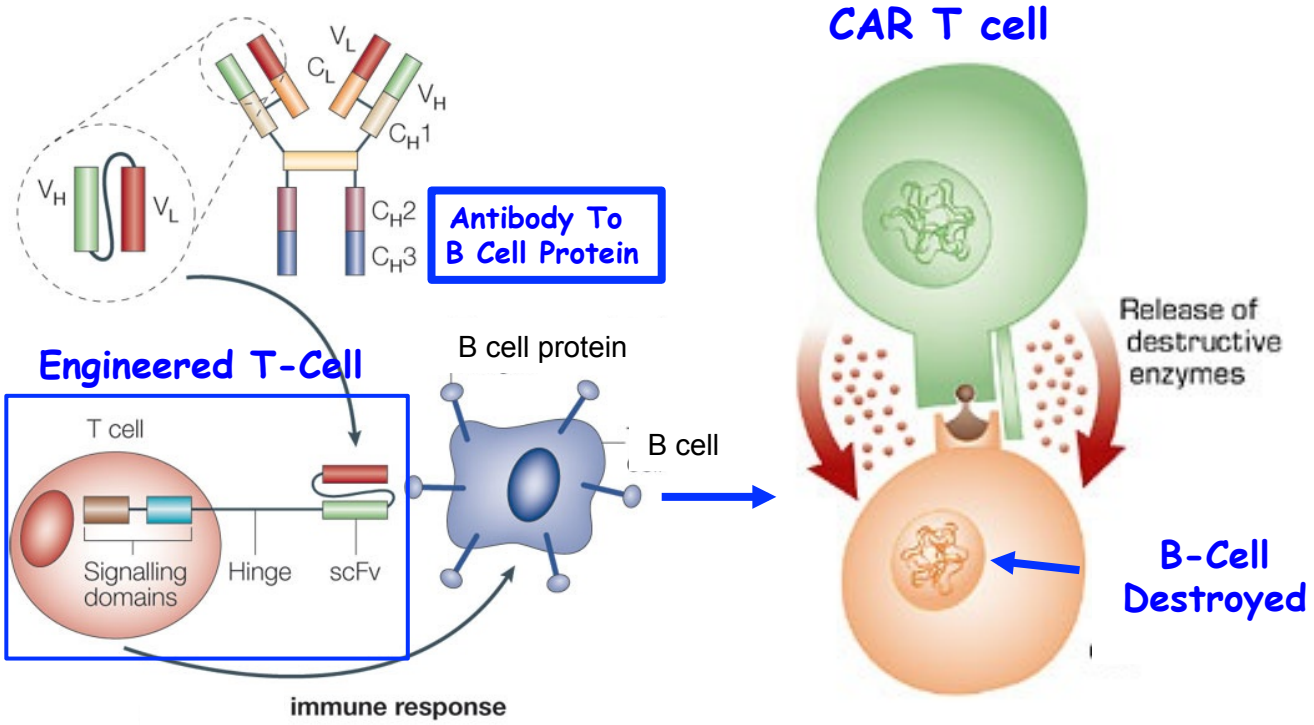
Engineering Chimeric Antigen Receptor Gene Tumor Cells T-Cells (CAR-T)

The Antigen is Specific For the Tumor and Binds to Tumor Receptors on Cell Surface





Retrovirus *Ex-Vivo* Gene Therapy for Chromic Lymphocytic Leukemia (CLL)



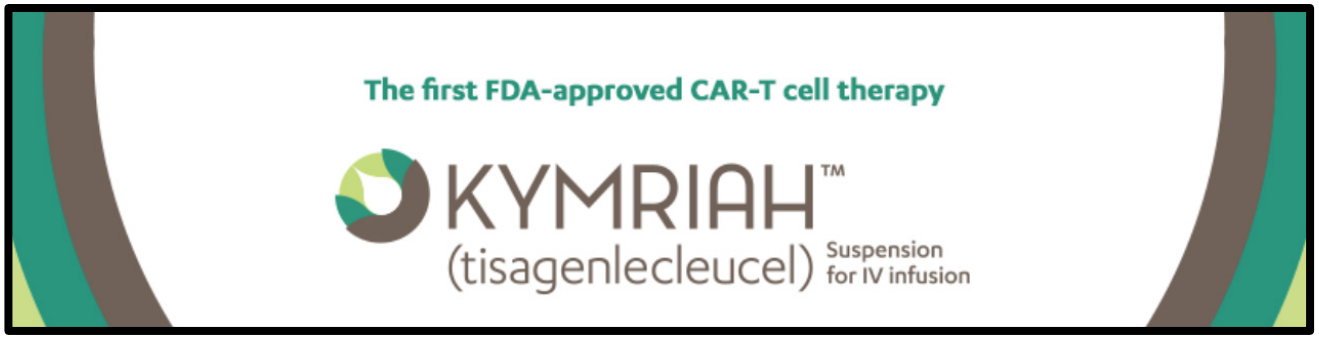
Protocol

1. Removed T Cells From Patients
2. Engineer Gene Encoding Chimeric Antigen Receptor (CAR) That Recognizes A Protein On The Surface Of B Cells
3. Transferred CAR Gene Into T Cells To Allow Them To Target B Cells
4. Infused CAR T Cells Back Into Patients

Results

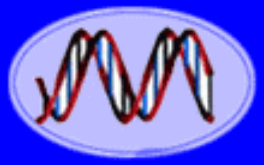
1. CAR T Cells Expanded More Than 1,000 Fold And Persisted More Than Six Months
2. Estimated That Each CAR T Cell Killed More Than 1,000 Cancer Cells
3. In One Trial, 19 Of 22 Children Who Had Exhausted All Drug Treatment And Bone-Marrow Transplant Options For Leukemia Went Into Remission After Receiving CART-19
4. 45 Of 75 Leukemia Patients Saw Complete Regressions With Cars

Two CAR-T Cell Gene Therapies Treatments Have Been by the FDA



- 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



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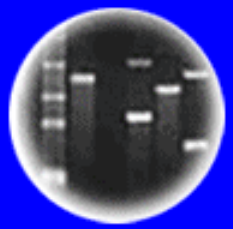

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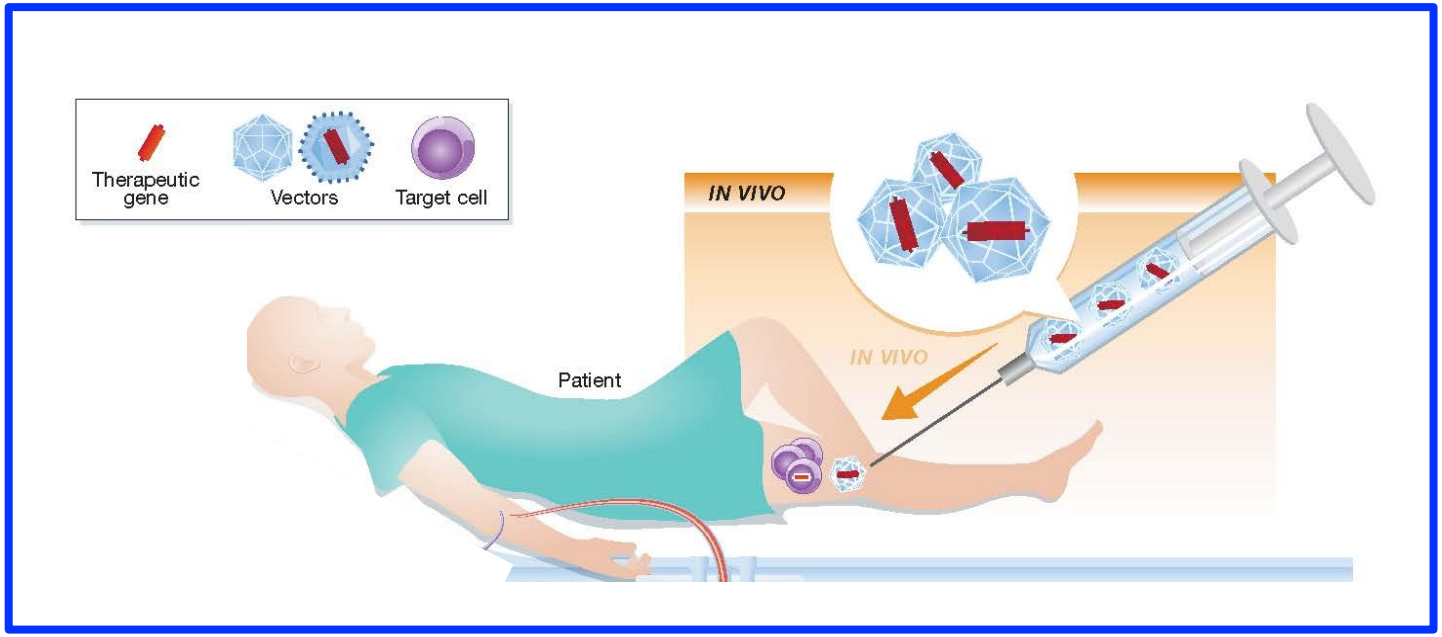
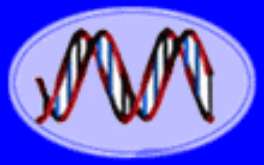
In Vivo Gene Therapy Using Viral Vectors



Replacement of Mutant Recessive Genes



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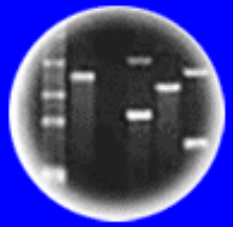

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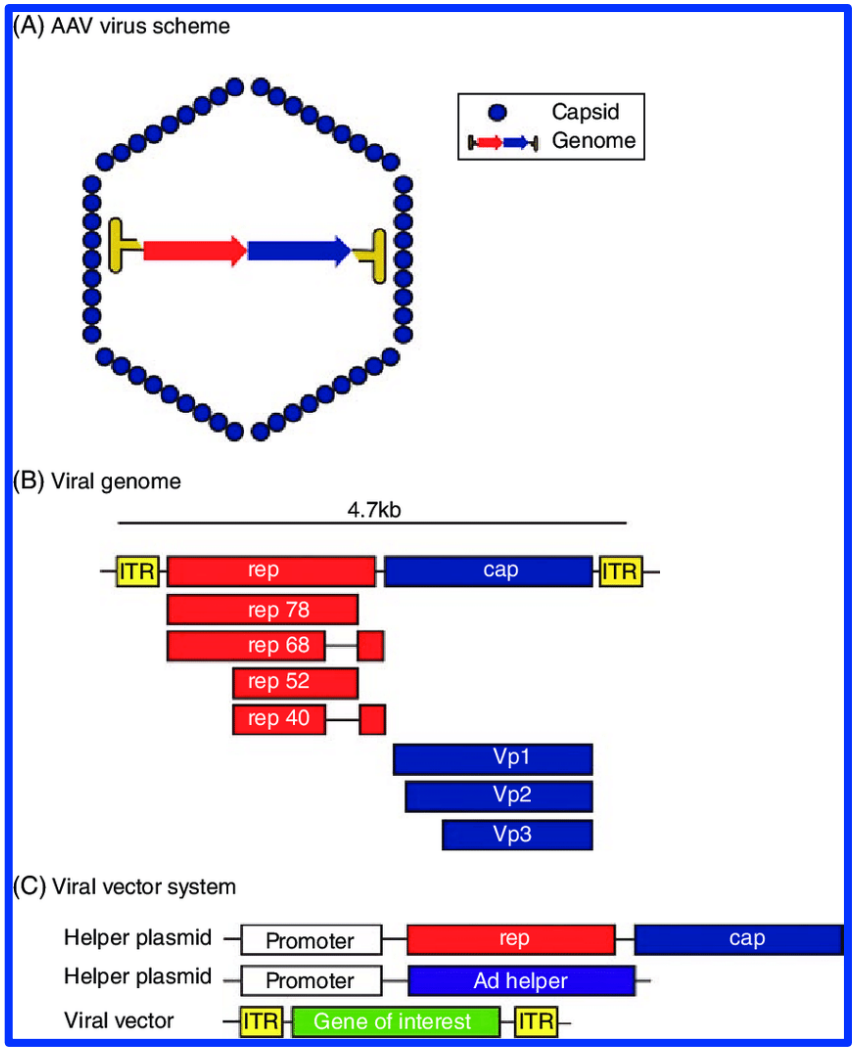
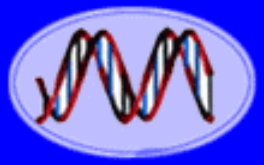
Adeno-Associated Viruses Are Viruses that Have a 5kb Single Stranded Genome, Infect a Wide Range of Cell Types, and Are Excellent Vectors For *In Vivo* Gene Therapy



Replacement of Defective Recessive Genes



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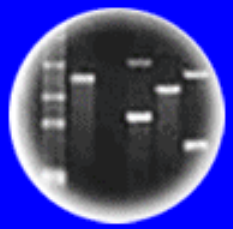

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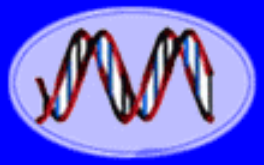
Adeno-Associated Viruses Infect a Wide Range of Cell Types…….

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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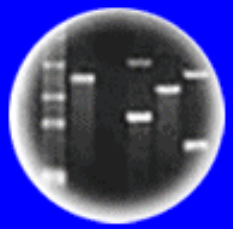

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Many Different Genetic Diseases Are Being Treated With *In Vivo* Gene Therapy

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

Hemophilia A & B Gene Therapy

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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]

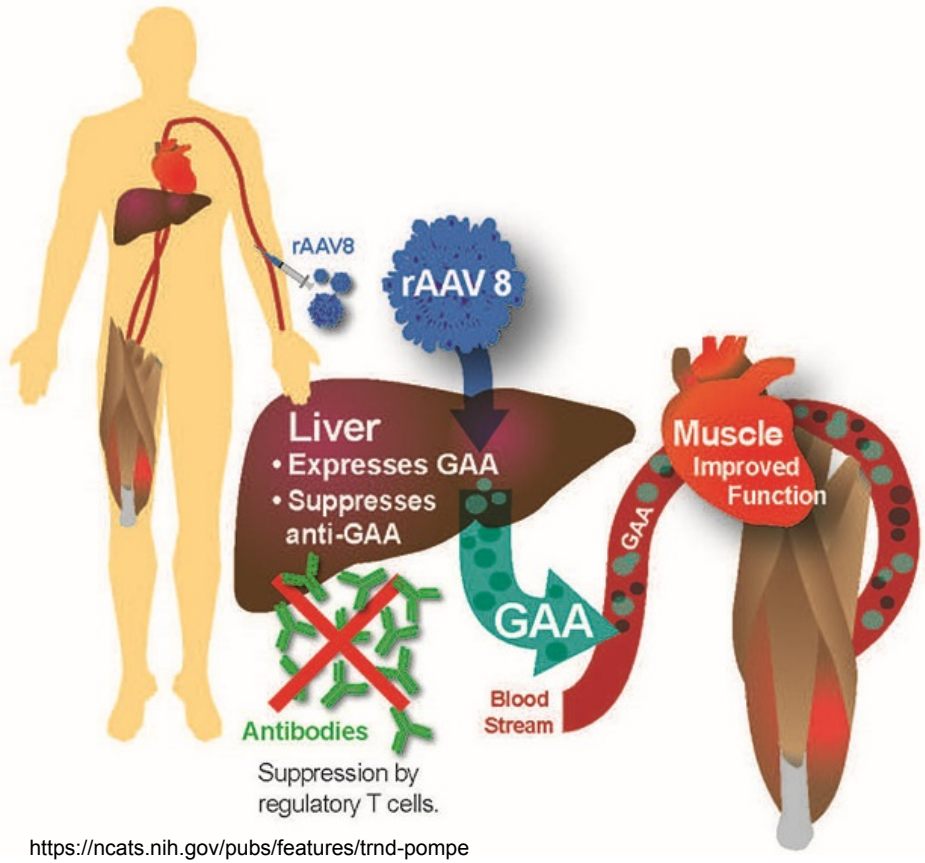
[1]Department of Pediatrics, Division of Cellular and Molecular Therapy, University of Florida, Gainesville, FL; and [2]Department of Pediatrics, Herman B Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, IN

In contrast to other diverse therapies for the X-linked bleeding disorder hemophilia that are currently in clinical development, gene therapy holds the promise of a lasting cure with a single drug administration. Near-to-complete correction of hemophilia A (factor VIII deficiency) and hemophilia B (factor IX deficiency) have now been achieved in patients by hepatic in vivo gene transfer. Adeno-associated viral vectors with different viral capsids that have been engineered to express high-level, and in some cases hyperactive, coagulation factors were employed. Patient data support that sustained endogenous production of clotting factor as a result of gene therapy eliminates the need for infusion of coagulation factors (or alternative drugs that promote coagulation), and may therefore ultimately also reduce treatment costs. However, mild liver toxicities have been observed in some patients receiving high vector doses. In some but not all instances, the toxicities correlated with a T-cell response directed against the viral capsid, prompting use of immune suppression. In addition, not all patients can be treated because of preexisting immunity to viral capsids. Nonetheless, studies in animal models of hemophilia suggest that the approach can also be used for immune tolerance induction to prevent or eliminate inhibitory antibodies against coagulation factors. These can form in traditional protein replacement therapy and represent a major complication of treatment. The current review provides a summary and update on advances in clinical gene therapies for hemophilia and its continued development. (*Blood*. 2019;133(5):407-414)

Companies sponsoring hemophilia gene therapy clinical trials

BIOMARIN CELEBRATING 20 YEARS · Pfizer · Sangamo THERAPEUTICS · SANOFI · Shire · Spark THERAPEUTICS · ultragenyx pharmaceutical · uniQure

Pompe Disease



https://ncats.nih.gov/pubs/features/trnd-pompe

POMPE DISEASE NEWS

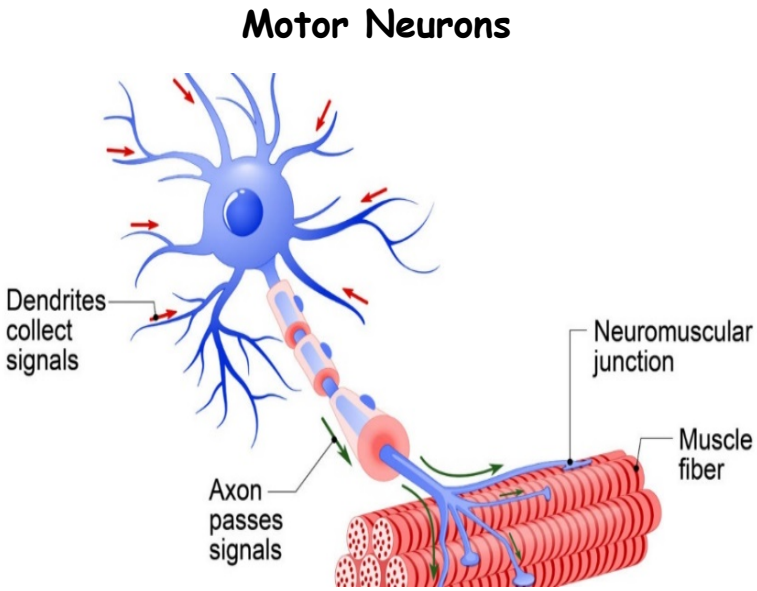
Actus Therapeutics

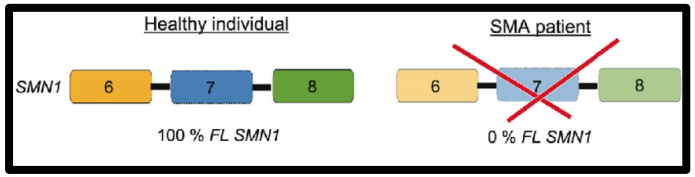
Phase 1/2 Trial Investigating ACTUS-101 Gene Therapy for Pompe Disease Doses First Patient

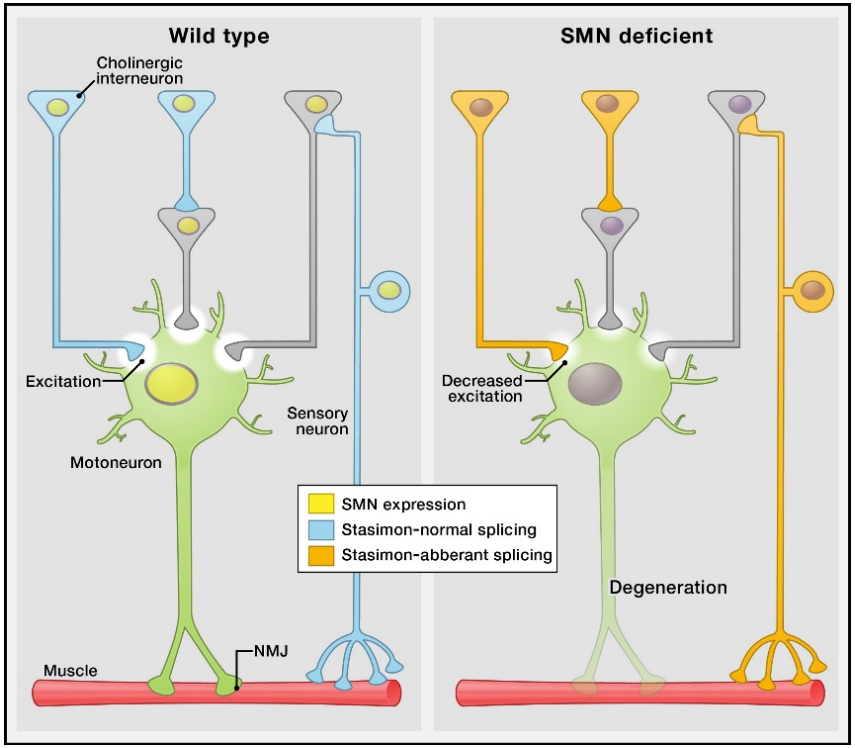
FEBRUARY 6, 2019 BY PATRICIA INACIO, PHD IN NEWS.

Spinal Muscular Atrophy (SMA) Gene Therapy

- 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







FDA Approves Gene Therapy for Spinal Muscular Atrophy

Spinal Muscular Atrophy Gene Therapy

Protocol For Phase 1 Clinical Trial

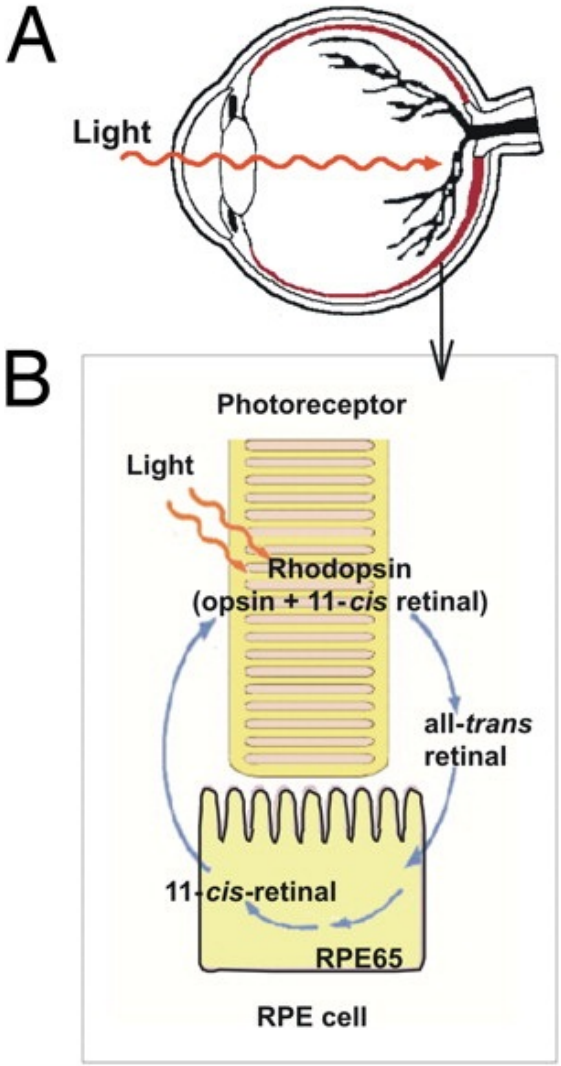
- Transferred The SMN Gene Into The AAV9 Vector
  - AAV9 When Infused Into A Vein Can Move Across The Blood-brain Barrier To The Central Nervous System
- Patients Were Given A Single Of Intravenous AAV9-SMN Treatment – 3 At A Low Dose And 12 At A High Dose

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

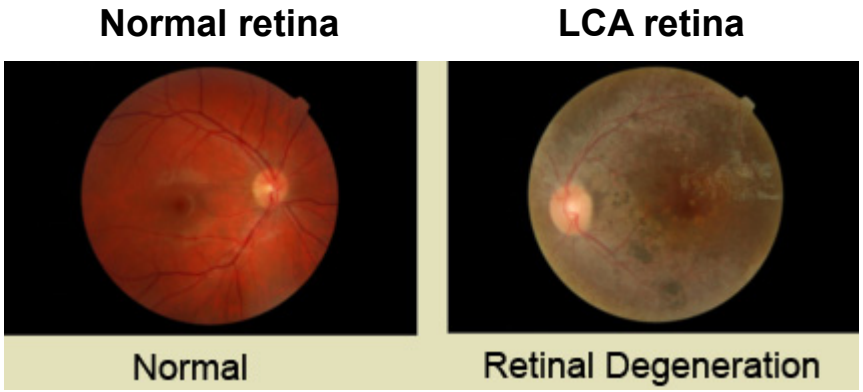
Blindness - Leber Congenital Amaurosis (LCA) Gene Therapy

How We See

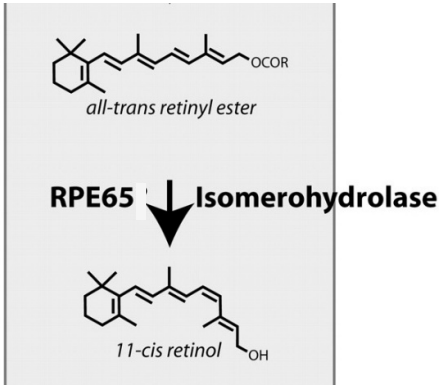


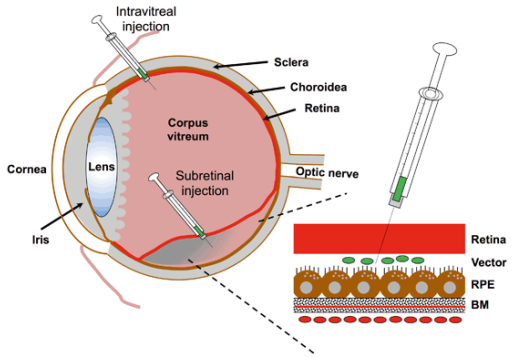
Cideciyan et al. PNAS 2008;105:15112

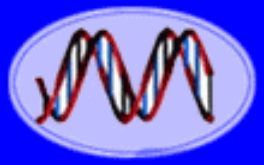
- Degenerative Disease Of The Retina
- The Most Common Cause Of Congenital Blindness In Children



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







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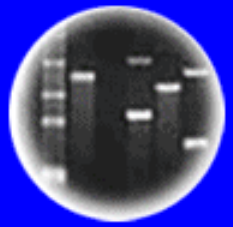

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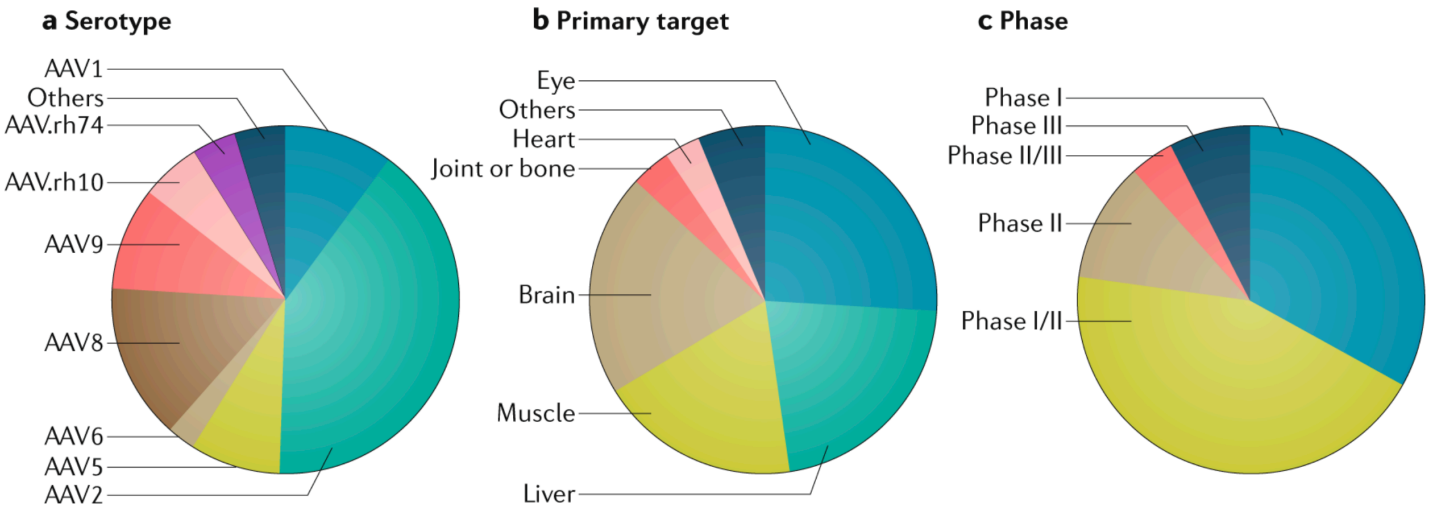
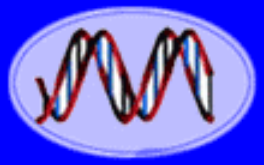
In Vivo Gene Therapy Clinical Trials With Adeno-Associated Viral Vectors

From: Adeno-associated virus vector as a platform for gene therapy delivery



The data set is from ClinicalTrials.gov, accessed on 13 November 2018. The 145 registered trials are categorized on the basis of adeno-associated virus (AAV) capsid serotype (part **a**), primary tissue target for gene delivery (part **b**) and



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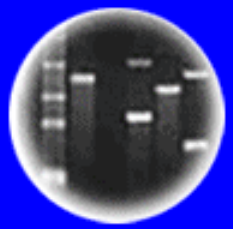
Genetic Code of Life

Entire Genetic Code of a Bacteria

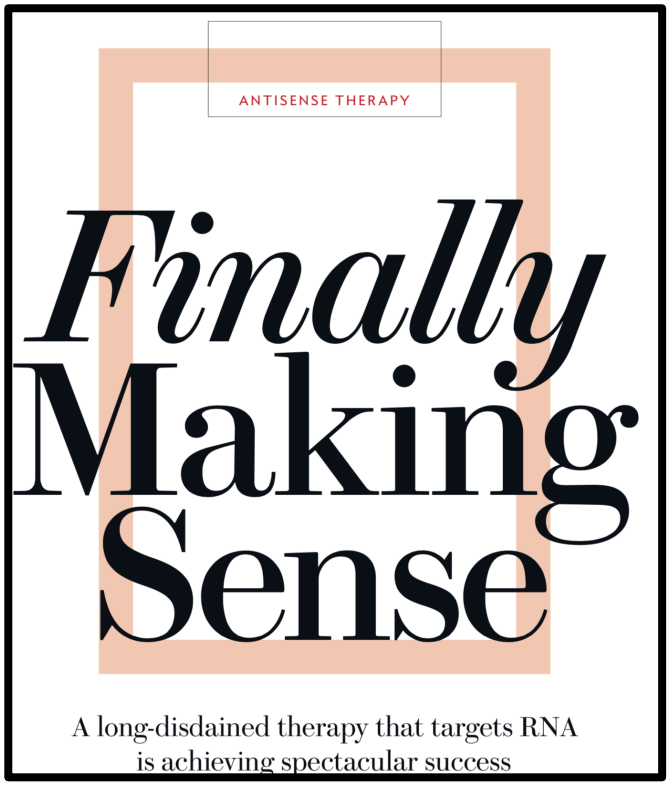


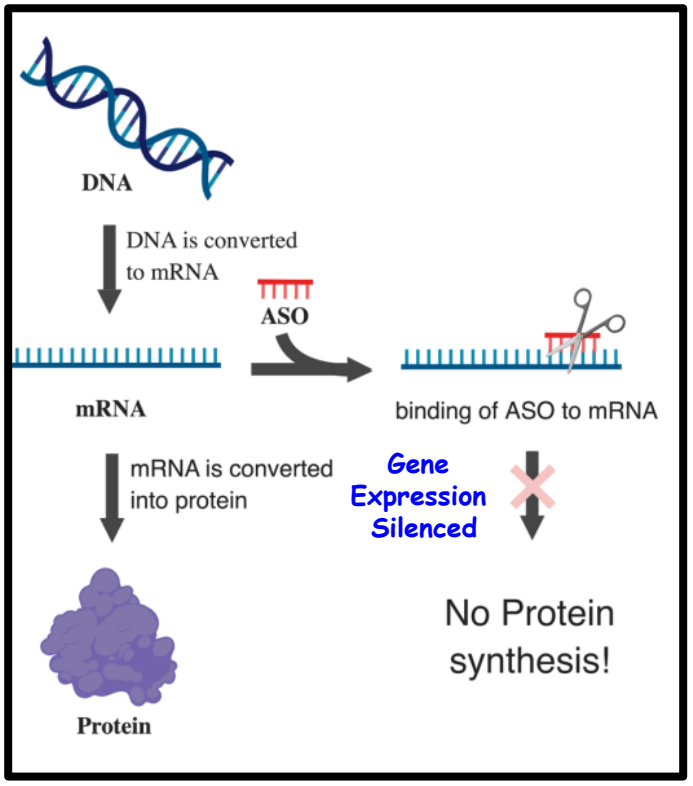
DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

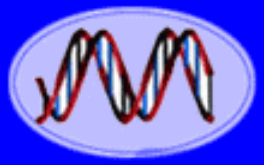
Plants of Tomorrow

Using ASOs and *In Vivo* Gene Therapy to Silence Gene Activity and Treat Dominant Genetic Disorders





Late Onset Genetic Diseases
Huntington's Disease
Lou Gehrig's Disease (ALS)



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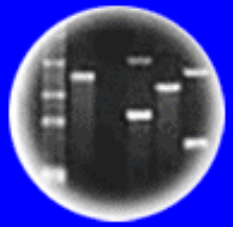

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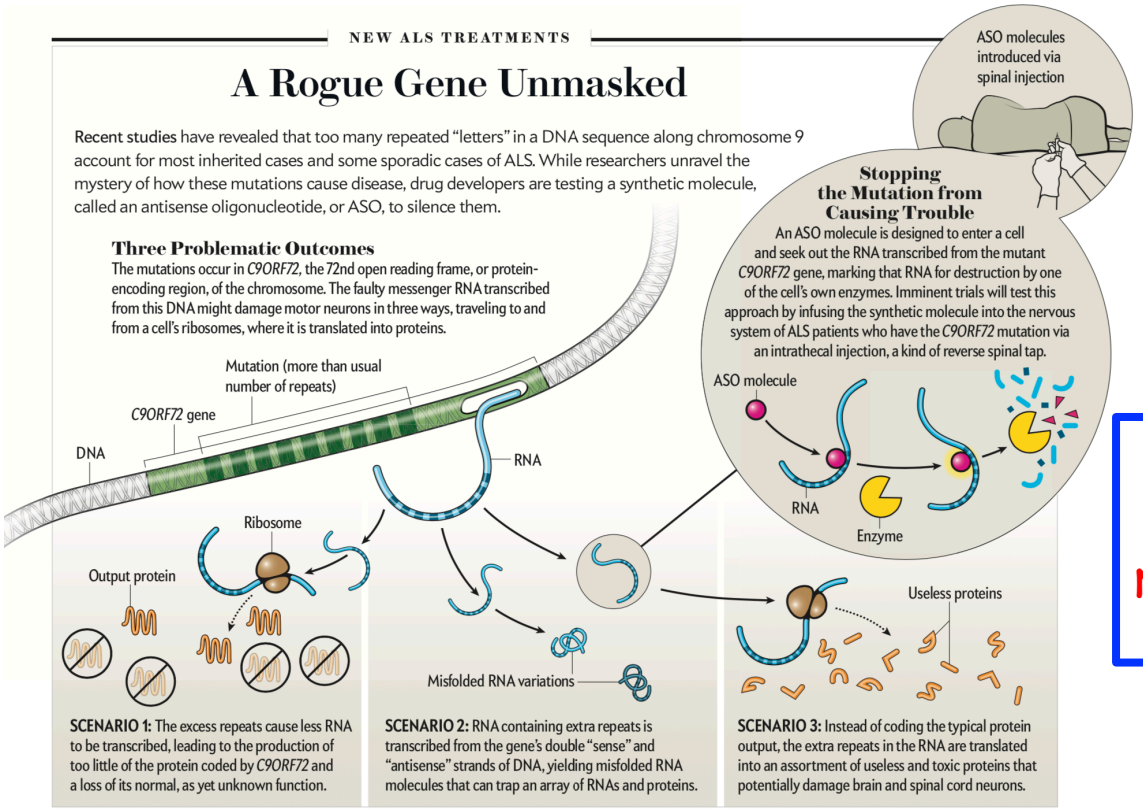
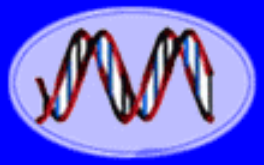
Plants of Tomorrow

Using ASOs and *In Vivo* Gene Therapy to Treat ALS or Lou Gehrig's Disease –A Dominant Genetic Disease



Toxic ALS Proteins Inhibit Normal ALS Proteins

Allele-Specific Oligonucleotide Anneals to Complementary mRNA Region Inhibiting mRNA translation and/or Marking mRNA for Destruction Recessive Normal Allele Can Now Function Properly



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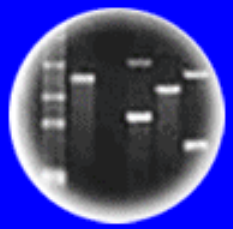


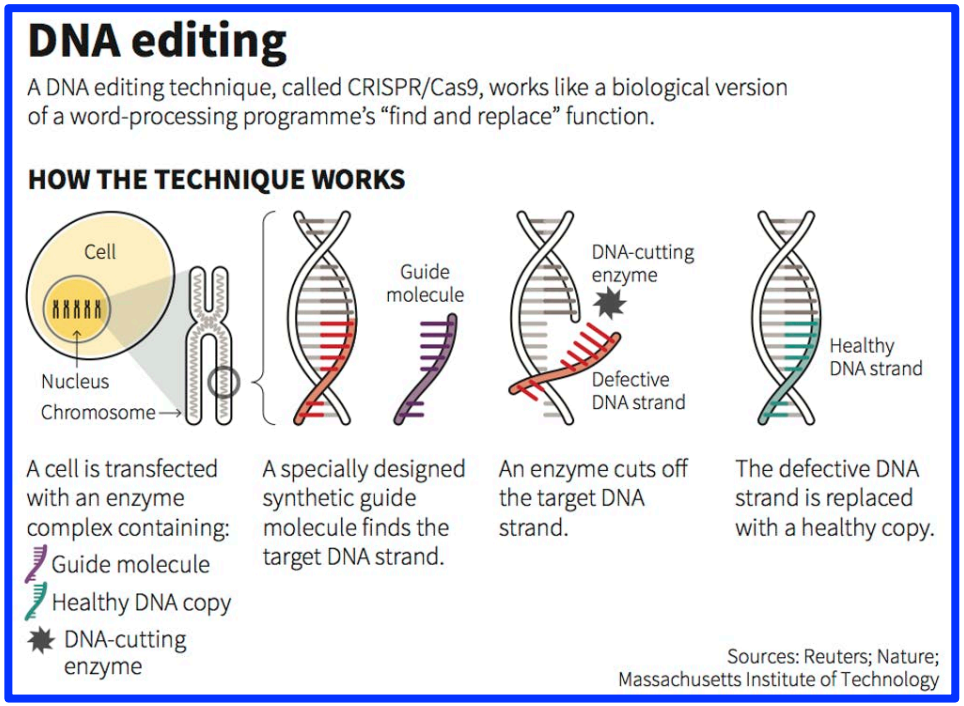
DNA Fingerprinting

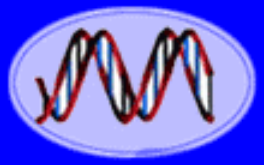
Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

Gene Editing & Human Gene Therapy

Dominant & Recessive Genes
Germline & Somatic Cell Gene Therapy
Editing Does it All!





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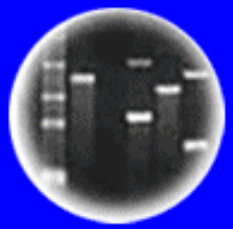
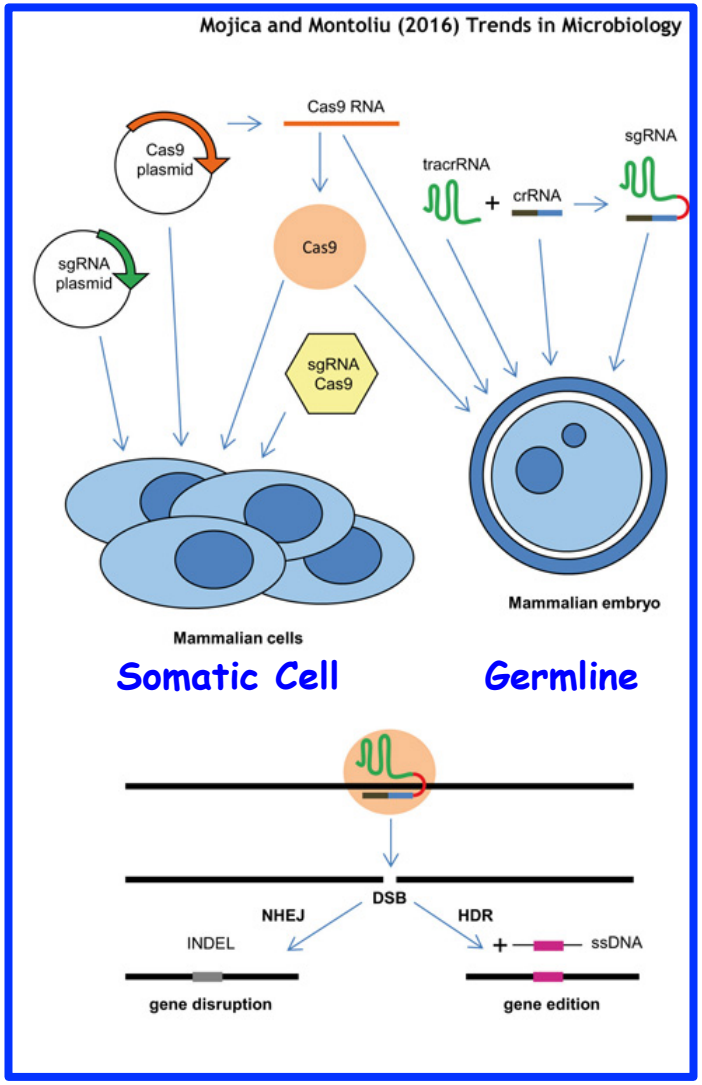


DNA Fingerprinting

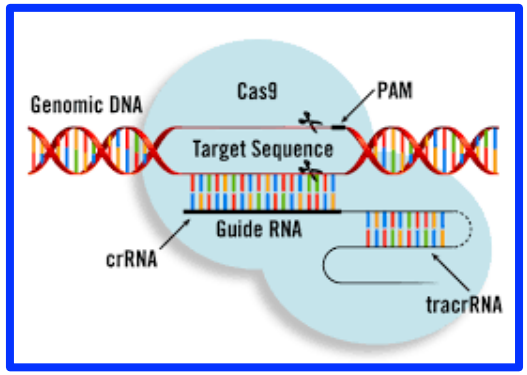
Cloning: Ethical Issues and Future Consequences

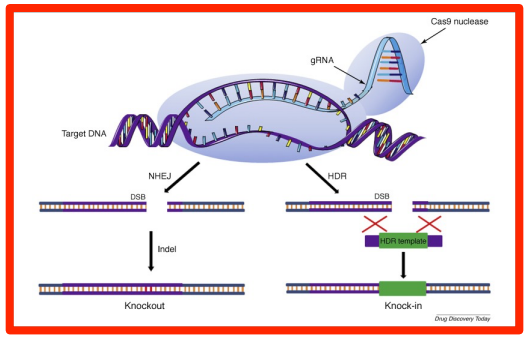
Plants of Tomorrow

How To Use the CRISPR-Cas System For Editing Specific Genes



1. Clone Cas9 & Guide RNAs
2. Transform Cells Using Relevant Vector
3. Edit Target Gene Sequence





Gene Editing Clinical Trials

NATIONAL HEMOPHILIA FOUNDATION
for all bleeding disorders

Sangamo Reports Positive Preliminary Data from Hemophilia Gene Therapy Trial

August 9, 2018

Gene Therapy for Hunter's Syndrome

TheScientist
EXPLORING LIFE, INSPIRING INNOVATION

NEWS & OPINION MAGAZINE SUBJECTS

Home / News & Opinion

Preliminary Results Point to Success of In Vivo Gene Editing

Two studies show signs that the introduced DNA is functioning, but it's too early to know if patients actually benefit.

Feb 12, 2019
CAROLYN WILKE

387 1 44

Brian Madeux – First Human Gene Editing Therapy Patient - 2018

US Regulatory Authority for Gene Therapy

- Department Of Health And Human Services Has Been Charged With Oversight Of Clinical Trials (DHHS)
  - Office For Human Research Protections
    - All Research Involving Human Subjects Undergo Institutional Review Board Review (IRB)
  - U.S. Food And Drug Administration (FDA)
    - 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 Oversees The Conduct Of Federally Funded Clinical Trials (NIH and RAC)
  - Recombinant DNA Advisory Committee (RAC) Review Human Gene Transfer Research On Behalf Of The NIH Through The Office Of Biotechnology Activities

Gene Therapy Comes of Age

The NEW ENGLAND JOURNAL of MEDICINE

Perspective

OCTOBER 11, 2018

The Next Phase of Human Gene-Therapy Oversight

Francis S. Collins, M.D., Ph.D., and Scott Gottlieb, M.D.

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

National Institutes of Health (NIH) Office of Science Policy (OSP) Recombinant or Synthetic Nucleic Acid Research: Proposed Changes to the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)

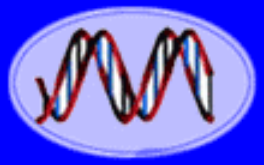
AGENCY: National Institutes of Health, HHS.

ACTION: Notice.

SUMMARY: The National Institutes of Health (NIH) seeks public comment on its proposal to amend the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)* to streamline oversight for human gene transfer clinical research protocols and reduce duplicative reporting requirements already captured within the existing regulatory framework. Specifically, NIH proposes amendments to: Delete the NIH protocol registration submission and reporting requirements under Appendix M of the *NIH Guidelines*, and modify the roles and responsibilities of entities that involve human gene transfer or the Recombinant DNA Advisory Committee (RAC).

DATES: To ensure consideration, comments must be submitted in writing by October 16, 2018.

In changes proposed on August 17, 2018, in the *Federal Register*, the NIH and the FDA seek to reduce the duplicative oversight burden by further limiting the role of the NIH and RAC in assessing gene-therapy protocols and reviewing their safety information. Specifically, these proposals will eliminate RAC review and reporting requirements to the NIH for human gene-therapy protocols. They will also revise the responsibilities of institutional Biosafety Committees, which have local oversight for this research, making their review of human gene-therapy protocols consistent with review of other research subject to the *NIH Guidelines*. Such streamlining will also appropriately place the focus of the *NIH Guidelines* squarely back on laboratory biosafety.



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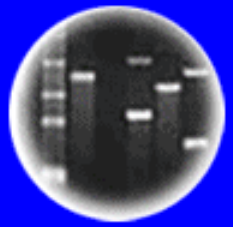

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Current Status of Gene Therapy

Approved Gene Therapy Products Worldwide

$10B Market By 2024

赛百诺 SIBIONO

P53 tumor suppressor deficiency
Marketed in China 2004

~~uniQure~~

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

Strimvelis

ADA-SCID
European Medicines Agency
Approved in 2016

LCA Blindness
FDA Approved 2017

LUXTURNA™
voretigene neparvovec-rzyl
for subretinal injection

NOW APPROVED

CAR-T Therapy
FDA Approved 2017

YESCARTA™
(axicabtagene ciloleucel) Suspension for IV infusion

Hereditary Transthyretin Amyloidosis
FDA Approved 2018

onpattro™
(patisiran) lipid complex injection

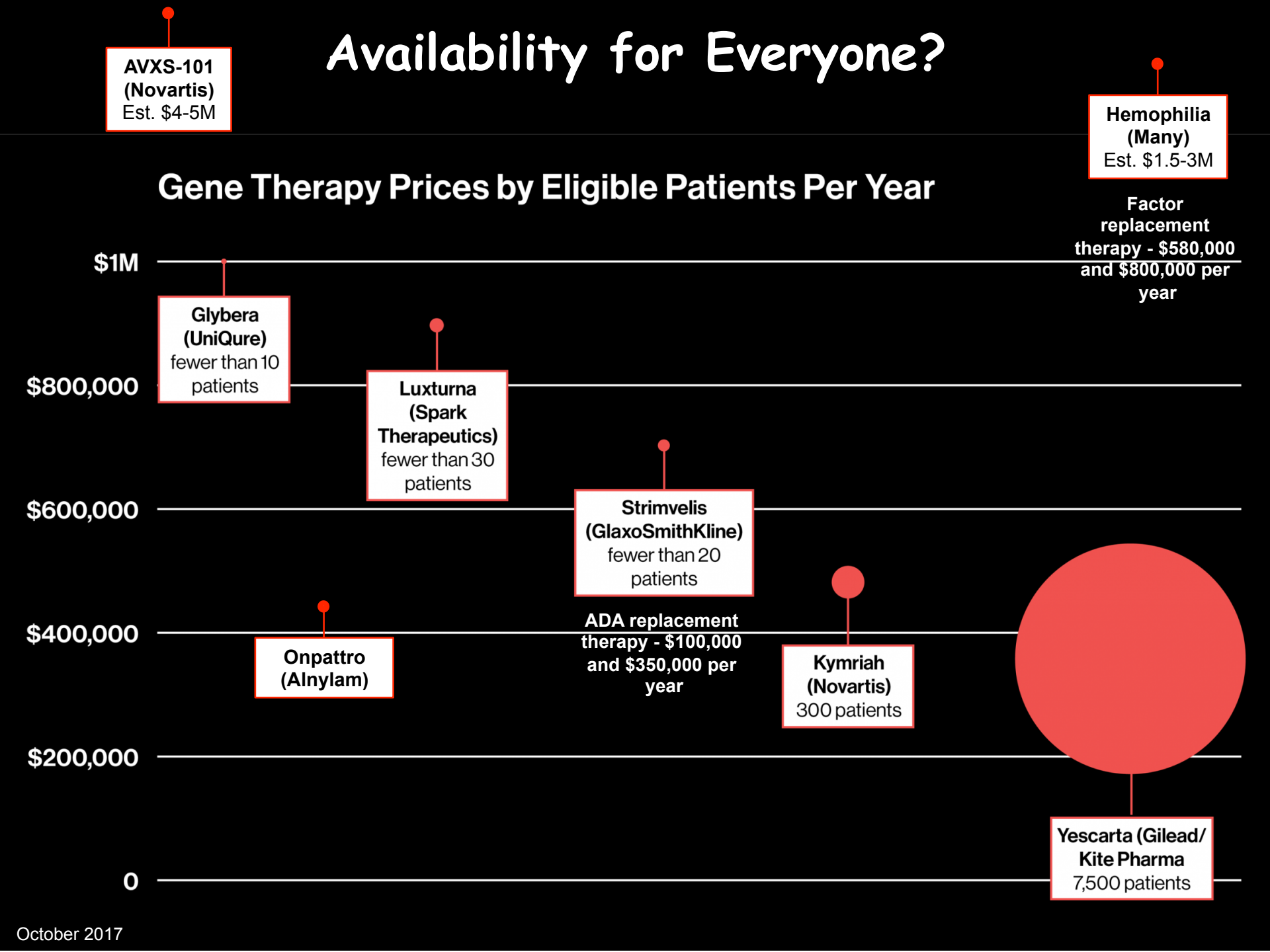
2017
Introducing the first
FDA-approved CAR-T cell therapy:
CTL019 is now

KYMRIAH™
(tisagenlecleucel) Suspension for IV infusion

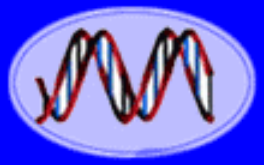
AVXS-101
avexis

Spinal Muscular Atrophy
Anticipated FDA Approval in
2019

Availability for Everyone?



October 2017



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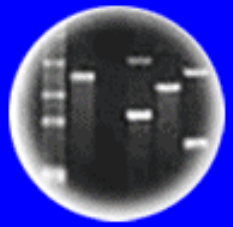

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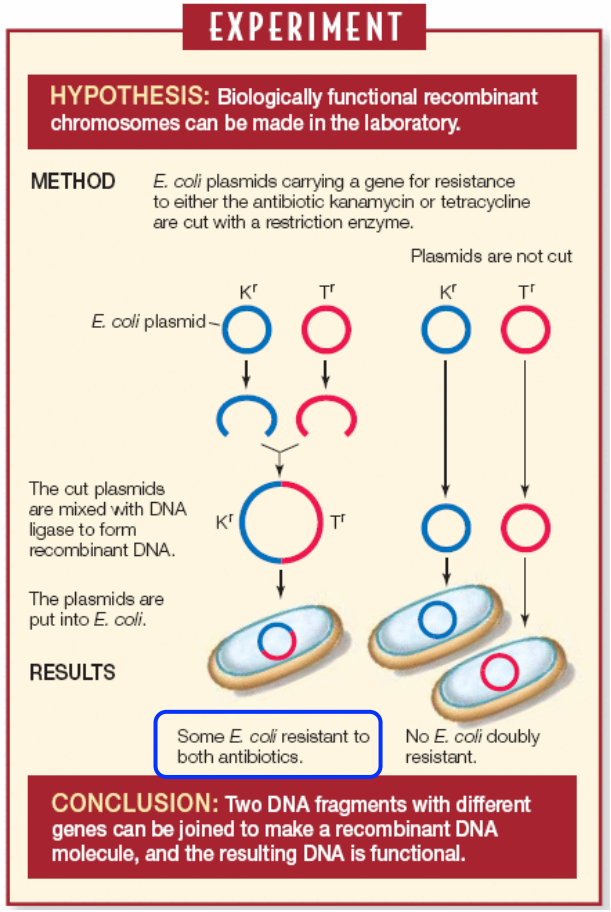
The End!!
Lectures on the History, Science, and Applications of Genetic Engineering……



…….On to Genetic Engineering & the Law