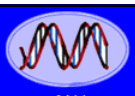


DNA
Genetic Code of Life

Entire Genetic Code of a Bacteria

DNA Fingerprinting

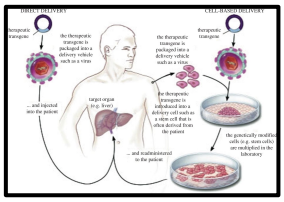
Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

HC70A
Spring 2020
Genetic Engineering in Medicine, Agriculture, and Law

Professor Bob Goldberg

Lecture 8
Human Genetic Engineering



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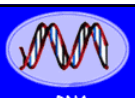

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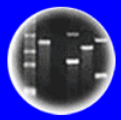

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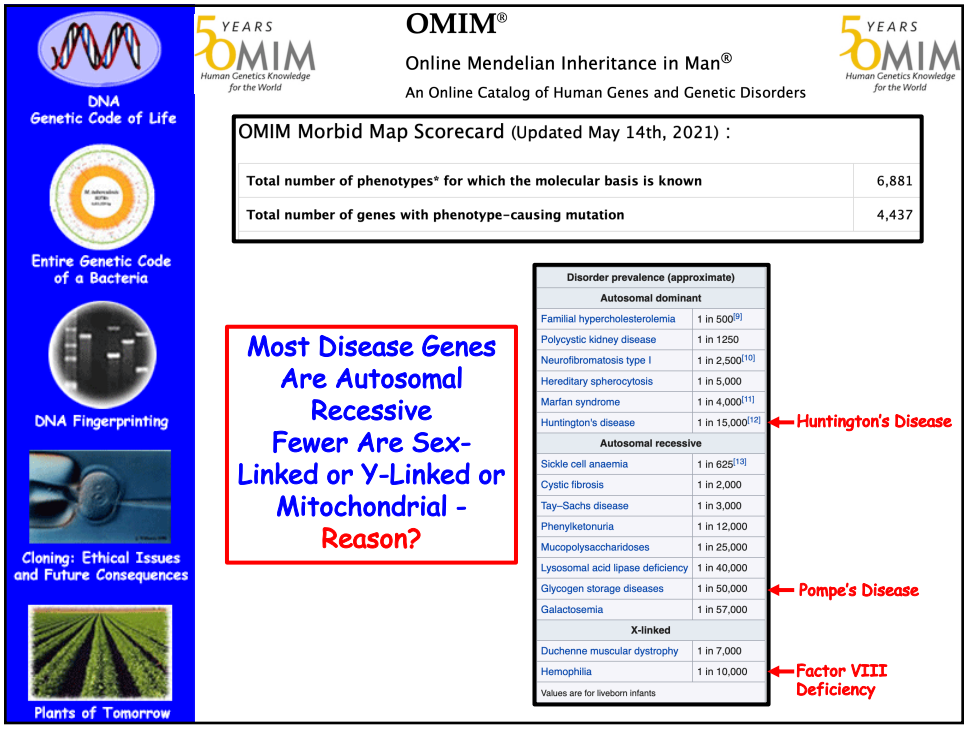
THEMES

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. In Vivo
    b. Ex 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
    e. Lentiviral Vectors
10. Ex Vivo Gene Therapy for Cancer – CAR-T
11. In Vivo Gene Therapy
12. ASO Gene Silencing For Dominant Genetic Disorders
13. Gene Editing
14. Regulation of Gene Therapy and Gene Therapy Landscape

2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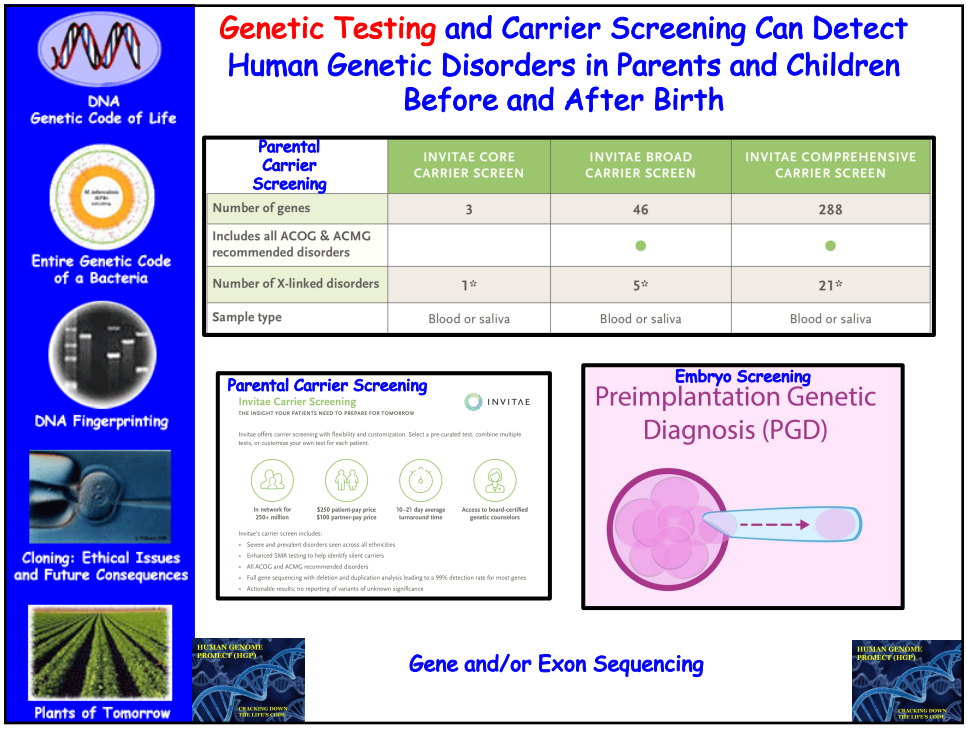
5 YEARS OMIM Human Genetics Knowledge for the World
OMIM®
Online Mendelian Inheritance in Man®
An Online Catalog of Human Genes and Genetic Disorders
5 YEARS OMIM Human Genetics Knowledge for the World
OMIM Morbid Map Scorecard (Updated May 14th, 2021) :
Total number of phenotypes* for which the molecular basis is known 6,881
Total number of genes with phenotype-causing mutation 4,437
Most Disease Genes Are Autosomal Recessive
Fewer Are Sex-Linked or Y-Linked or Mitochondrial - Reason?
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]
Huntington's Disease
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
Pompe's Disease
Galactosemia 1 in 57,000
X-linked
Duchenne muscular dystrophy 1 in 7,000
Hemophilia 1 in 10,000
Factor VIII Deficiency
Values are for liveborn infants

3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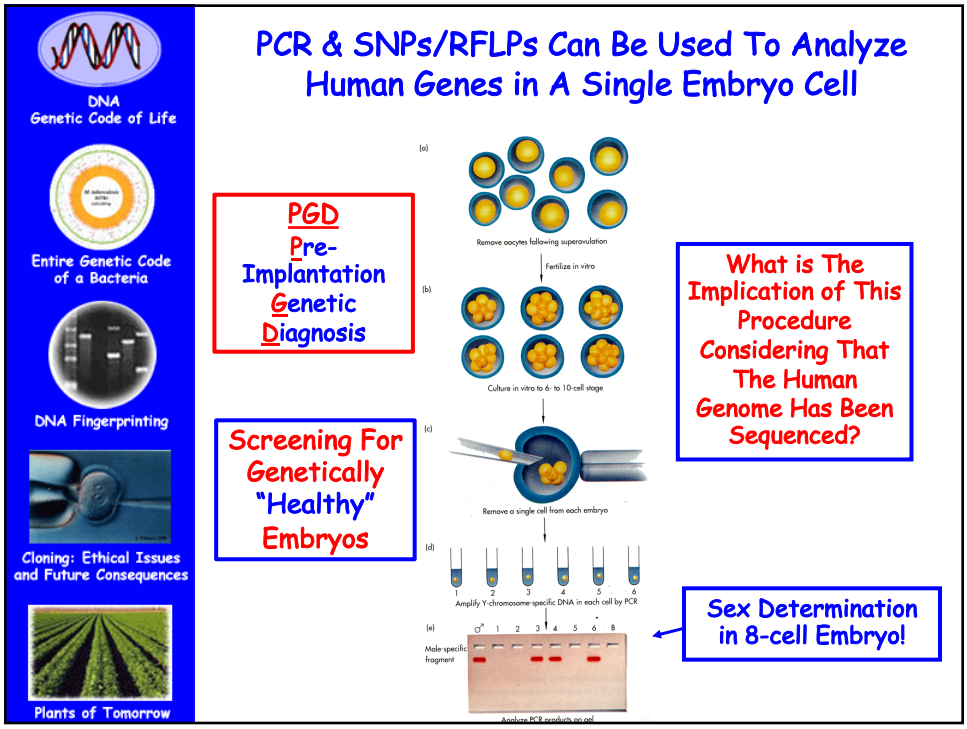
Genetic Testing and Carrier Screening Can Detect Human Genetic Disorders in Parents and Children Before and After Birth
Parental Carrier Screening
INVITAE CORE CARRIER SCREEN
INVITAE BROAD CARRIER SCREEN
INVITAE COMPREHENSIVE CARRIER SCREEN
Number of genes 3 46 288
Includes all ACOG & ACMG recommended disorders ● ●
Number of X-linked disorders 1* 5* 21*
Sample type Blood or saliva Blood or saliva Blood or saliva
Parental Carrier Screening
Invitae Carrier Screening
INVITAE
THE INSIGHT YOUR PATIENTS NEED TO PREPARE FOR TOMORROW
Invitae offers carrier screening with flexibility and customization. Select a pre-curated test, combine multiple tests, or customize your own test for each patient.
In network for 250+ million
$250 patient-pay price $100 partner-pay price
10–21 day average turnaround time
Access to board-certified genetic counselors
Invitae's carrier screen includes:
Severe and prevalent disorders seen across all ethnicities
Enhanced SMA testing to help identify silent carriers
All ACOG and ACMG recommended disorders
Full gene sequencing with deletion and duplication analysis leading to a 99% detection rate for most genes
Actionable results; no reporting of variants of unknown significance
Embryo Screening
Preimplantation Genetic Diagnosis (PGD)
Gene and/or Exon Sequencing
HUMAN GENOME PROJECT (HGP)
CRACKING DOWN THE LIFE'S CODE
HUMAN GENOME PROJECT (HGP)
CRACKING DOWN THE LIFE'S CODE

4

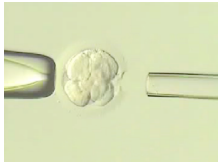


5

Determining the Genetic Identity of a Human Embryo Before Implantation!

Prenatal Genetic Diagnosis (PGD)

Fertility Clinics Scan for the Strongest Embryo



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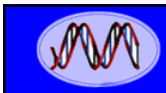

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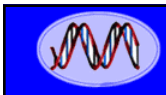
And Newborn Babies Are Screened For Many Genetic Diseases

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[1]	✓
	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™

7



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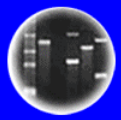

DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

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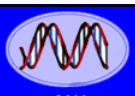
Treatments Have Been Developed For Genetic Diseases We Have Discussed in HC70A

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

8



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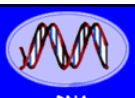
What Are the Prospects For a Permanent "Cure" For Genetic Diseases Using Gene Therapy?

9



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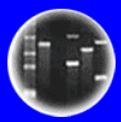

DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

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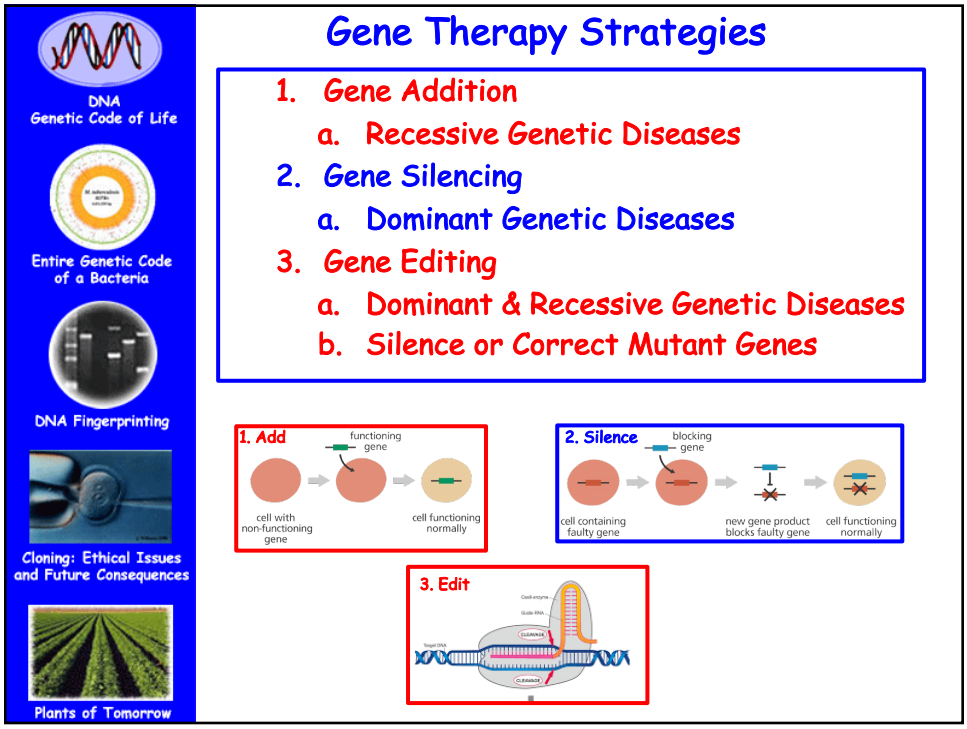
What Information is Needed Before Initiating Development of a Gene Therapy?

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?

http://learn.genetics.utah.edu

10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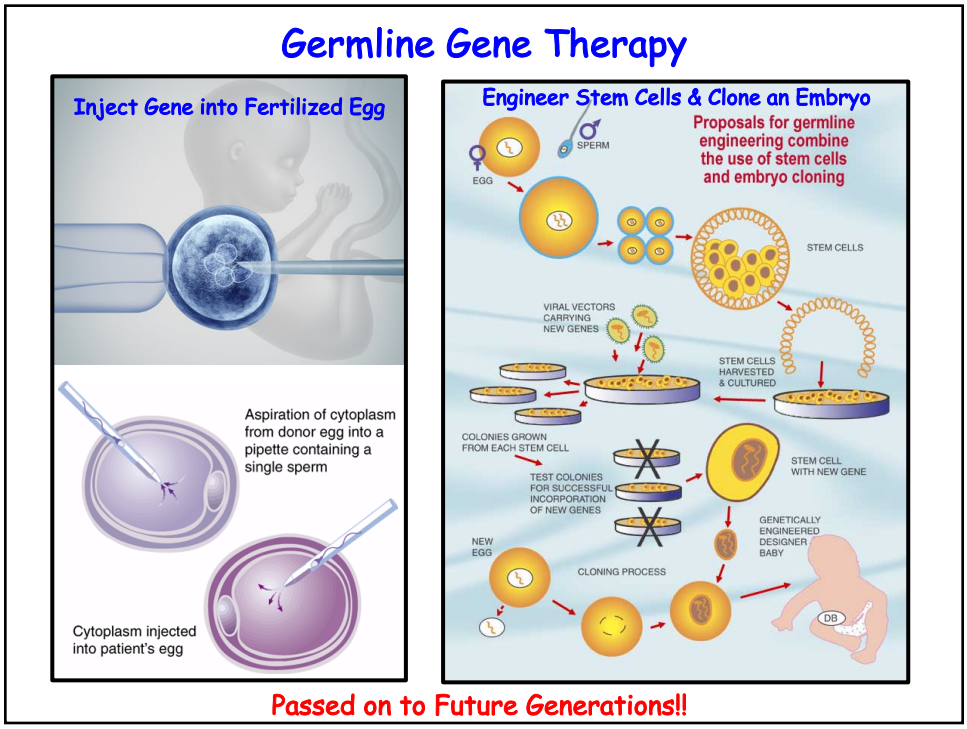
Gene Therapy Strategies
1. Gene Addition
a. Recessive Genetic Diseases
2. Gene Silencing
a. Dominant Genetic Diseases
3. Gene Editing
a. Dominant & Recessive Genetic Diseases
b. Silence or Correct Mutant Genes
1. Add
functioning gene
cell with non-functioning gene
cell functioning normally
2. Silence
blocking gene
cell containing faulty gene
new gene product blocks faulty gene
cell functioning normally
3. Edit

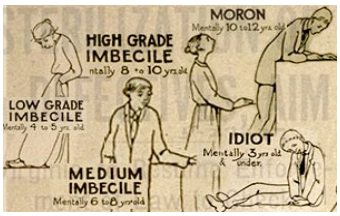
11


Germline Gene Therapy
Inject Gene into Fertilized Egg
Aspiration of cytoplasm from donor egg into a pipette containing a single sperm
Cytoplasm injected into patient's egg
Engineer Stem Cells & Clone an Embryo
Proposals for germline engineering combine the use of stem cells and embryo cloning
SPERM
EGG
STEM CELLS
VIRAL VECTORS CARRYING NEW GENES
STEM CELLS HARVESTED & CULTURED
COLONIES GROWN FROM EACH STEM CELL
TEST COLONIES FOR SUCCESSFUL INCORPORATION OF NEW GENES
STEM CELL WITH NEW GENE
GENETICALLY ENGINEERED DESIGNER BABY
NEW EGG
CLONING PROCESS
DB
Passed on to Future Generations!!

12

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

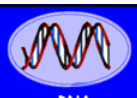
Some people are born to be a burden on the rest.




DECISION HELD
STEP TOWARD A SUPER-RACE
Health Official Praise Court in Upholding Sterilization Law
WASHINGTON, May 5.—(INS)—An improved race of Americans is on its

- 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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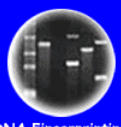
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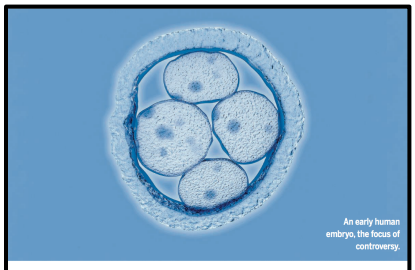

An early human embryo, the focus of controversy.

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

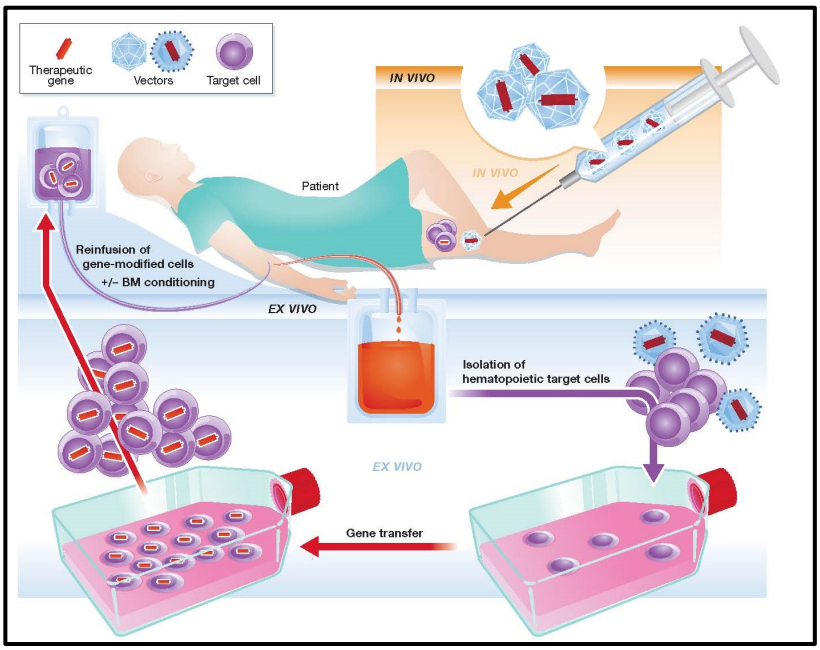
By NICHOLAS WADE MARCH 19, 2015

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

14

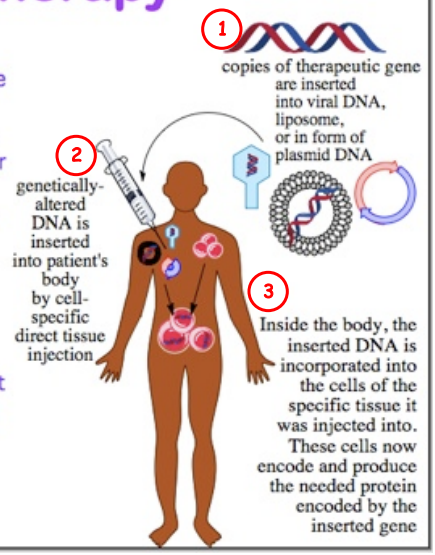
Somatic Cell Gene Therapy – *In Vivo* and *Ex Vivo*



15

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.



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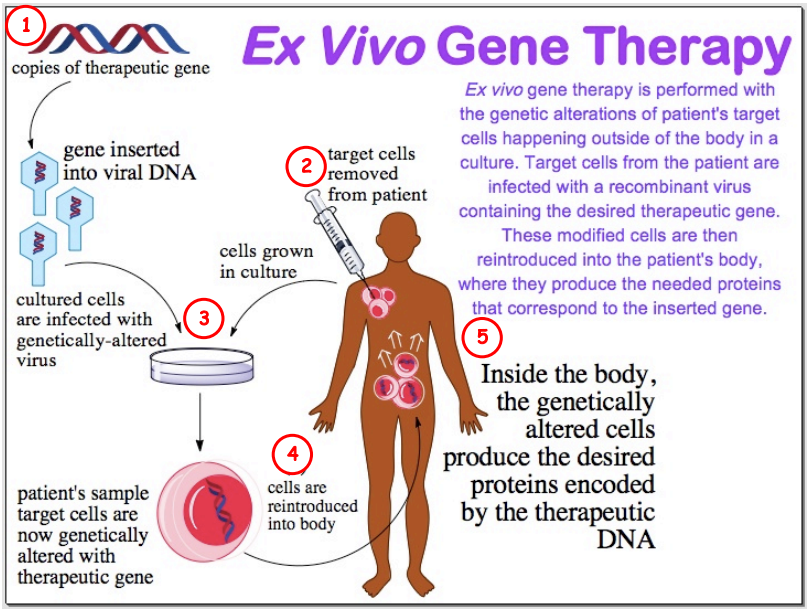

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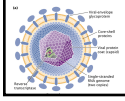
Viral Vectors Used to Deliver Genes to Cells in Somatic Cell Gene Therapy

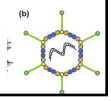
Table 1 Viral vectors discussed in this review

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





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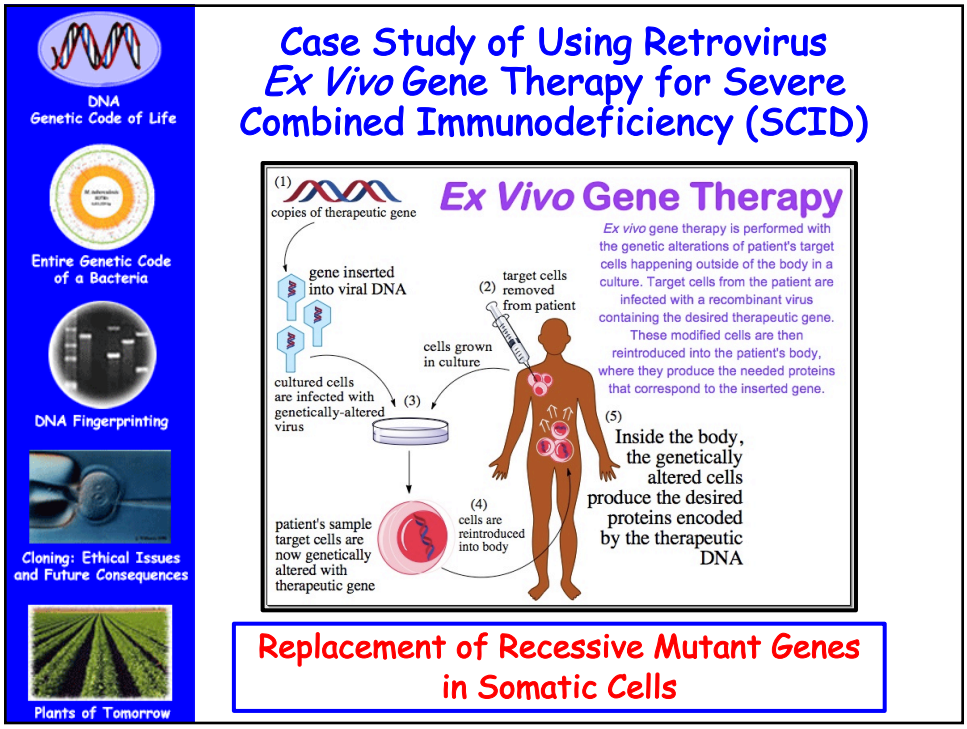
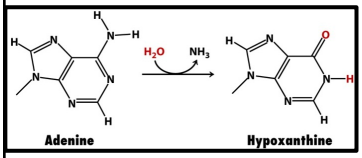


19

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

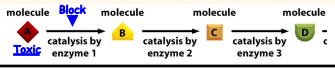
- 32,213 kb Gene
- Chromosome 20
- 12 Exons
- 1,092 kb mRNA
- 323 aa protein



Degradation of Adenosine

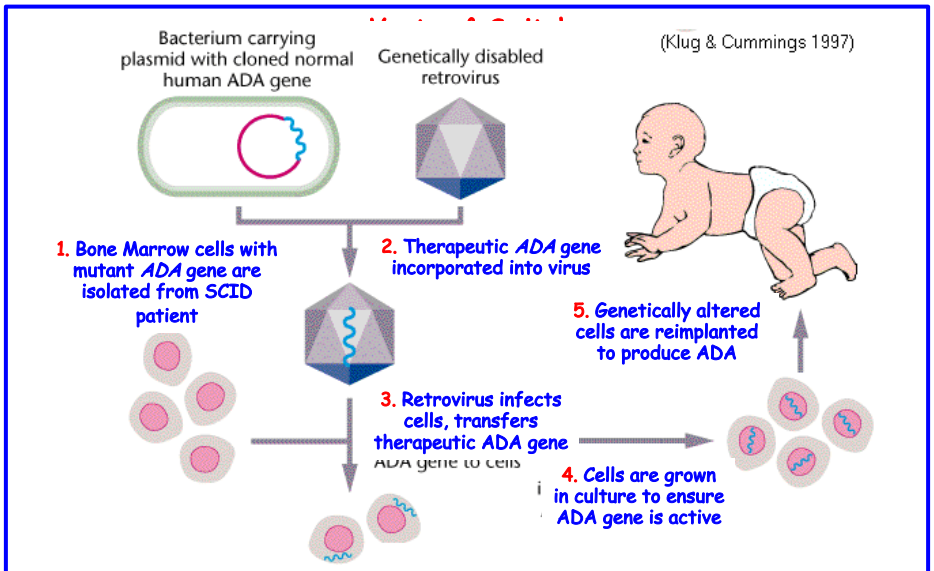
Treatments for ADA-SCID

	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**



20

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

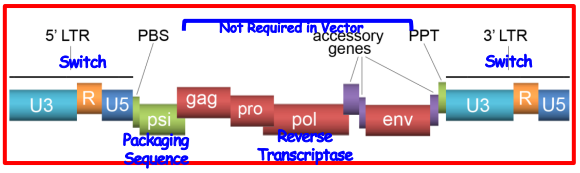


ADA-SCID Clinical Trial Started in 1990

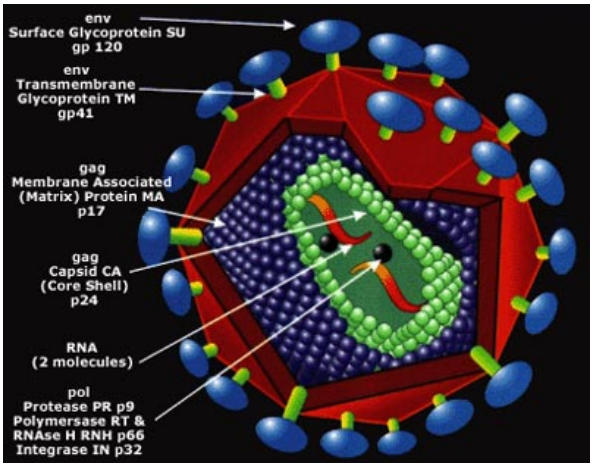
21

A Retrovirus and Its Genome

7-12 kb RNA Genome



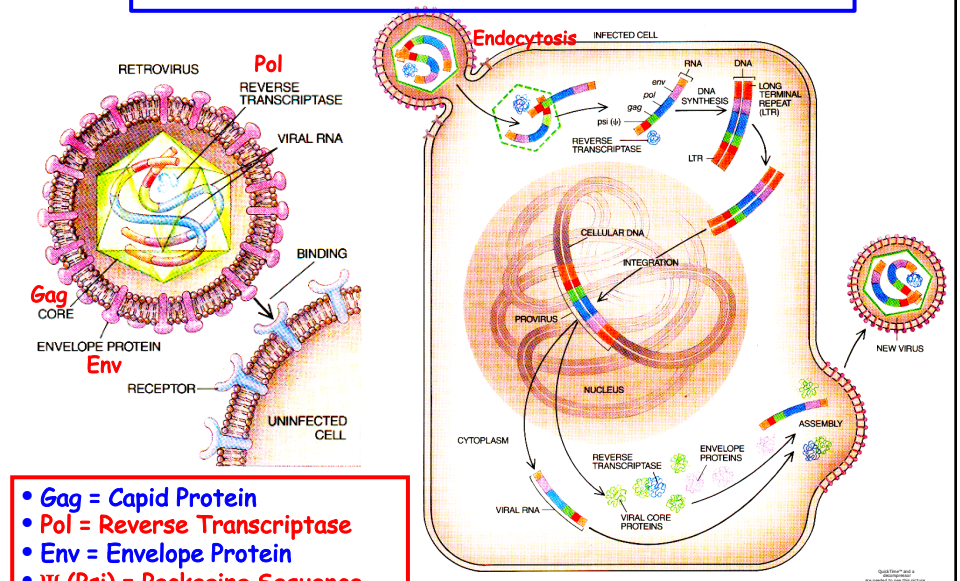
- 5' long terminal repeat (LTR) – strong switch & integration
- 3' LTR – strong switch, integration & transcriptional termination
- psi (Ψ) – packaging element needed to package the RNA genome into the viral particle
- gag – structural (coat) proteins
- pro – protease
- pol – reverse transcriptase
-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



22

Retrovirus Life Cycle

Retroviruses Integrate Their Genomes Into Human Cells

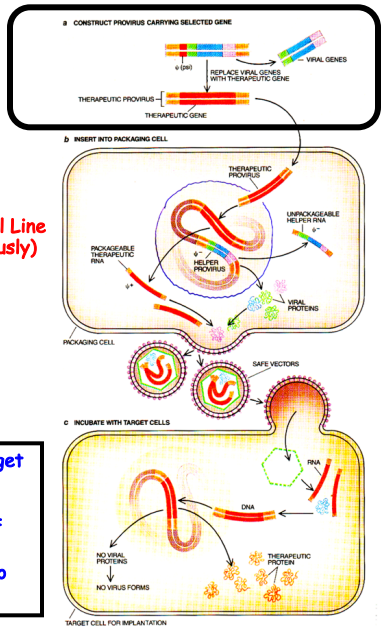


- Gag = Capid Protein
- Pol = Reverse Transcriptase
- Env = Envelope Protein
- Ψ (Psi) = Packaging Sequence
- LTR = Strong Switch

"Genetically Colonizes" Living Cells

23

Using Retroviruses for Ex Vivo Gene Therapy



Packaging Cell Line (Made Previously)

A.

1. Engineering 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.

24

Did the Gene Therapy Strategy Work?

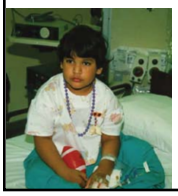
Science
AAAS

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

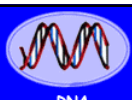


Ashanthi DeSilva 1992

Human "GMO"

Ashanthi DeSilva 2020

25



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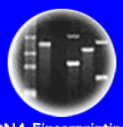
Entire Genetic Code of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

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)

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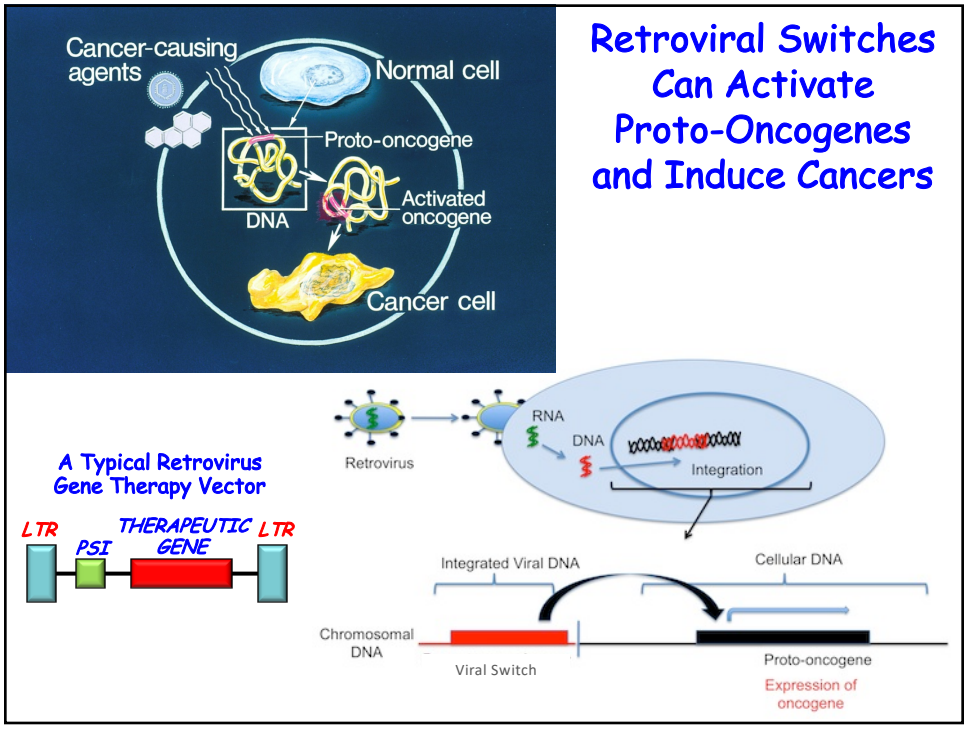
Cancer-causing agents
Normal cell
Proto-oncogene
DNA
Activated oncogene
Cancer cell
Retroviral Switches Can Activate Proto-Oncogenes and Induce Cancers
A Typical Retrovirus Gene Therapy Vector
LTR
PSI
THERAPEUTIC GENE
LTR
Retrovirus
RNA
DNA
Integration
Integrated Viral DNA
Cellular DNA
Chromosomal DNA
Viral Switch
Proto-oncogene
Expression of oncogene

27


DNA
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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 (AAV)

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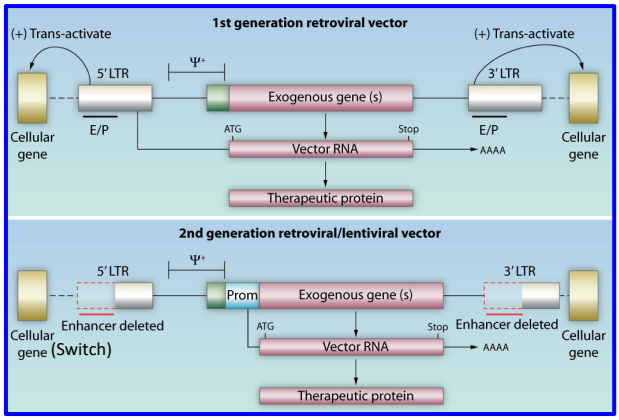
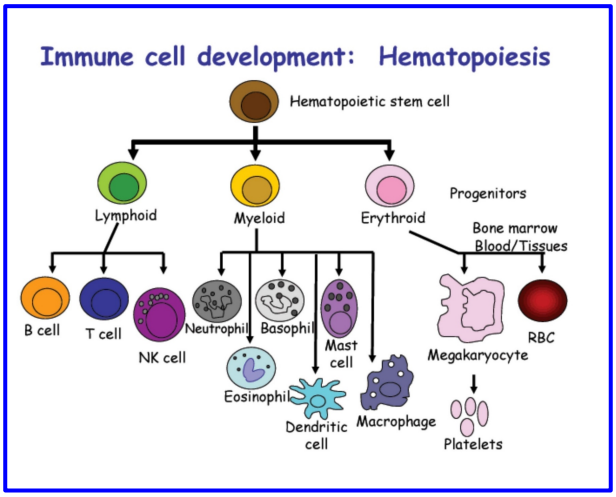
Development of Self-Inactivating (SIN) Lentiviral Vectors



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.

29

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

30

It Works!

Gene therapy cures 'bubble boy disease'

31 Jan 2009, 1128 hrs IST, AP

Strimvelis®

EMA APPROVED

The new england journal of medicine

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

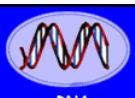
Gene Therapy for Immunodeficiency Due to Adenosine Deaminase Deficiency

Results After 10 Years

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

- 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

31



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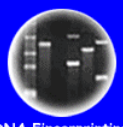

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The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

May 11, 2021

Autologous Ex Vivo Lentiviral Gene Therapy for Adenosine Deaminase Deficiency

Don Kohn - UCLA

Gene Therapy for ADA-SCID Still Benefitting Patients after Two to Three Years

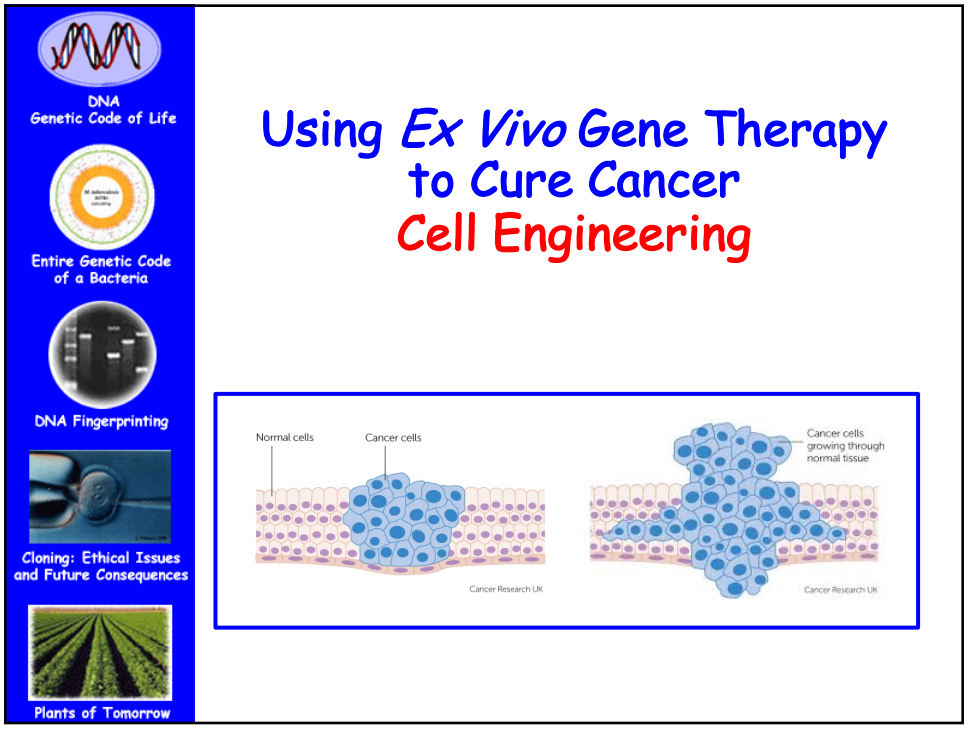
HEALTH + BEHAVIOR

Gene therapy offers potential cure to children born without an immune system

Study shows treatment developed by international team restored immune function in more than 95% of patients in three clinical trials

UCLA Broad Stem Cell Research Center

32


DNA
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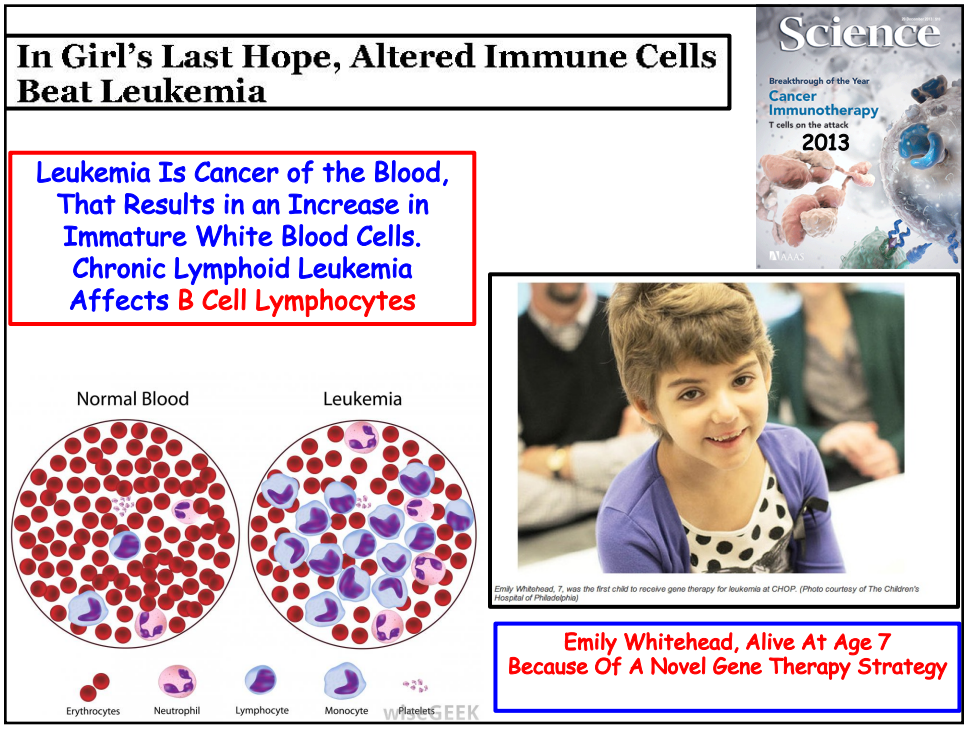
Cloning: Ethical Issues and Future Consequences
Plants of Tomorrow
Using Ex Vivo Gene Therapy to Cure Cancer
Cell Engineering
Normal cells
Cancer cells
Cancer Research UK
Cancer cells growing through normal tissue
Cancer Research UK

33


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
Science
Breakthrough of the Year
Cancer Immunotherapy
T cells on the attack
2013
Normal Blood
Leukemia
Erythrocytes
Neutrophil
Lymphocyte
Monocyte
Platelets
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

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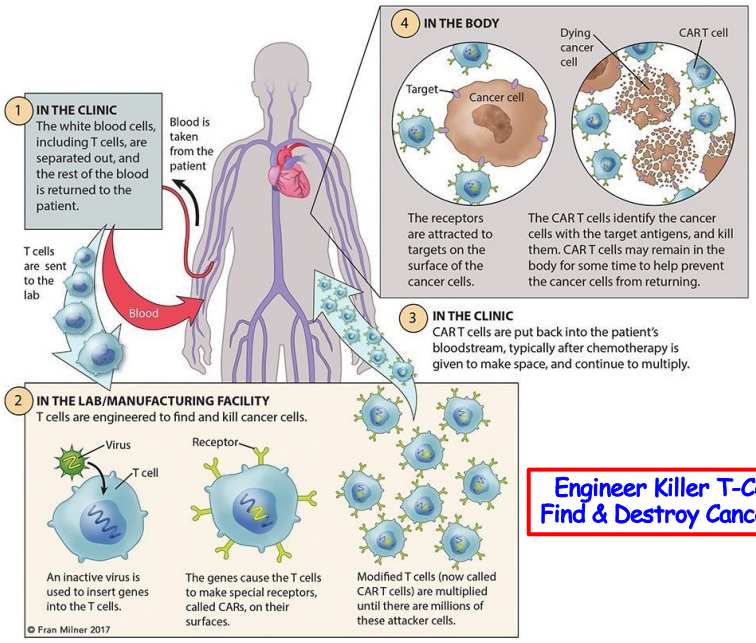
Chimeric Antigen Receptor (CAR-T) Cell Strategy



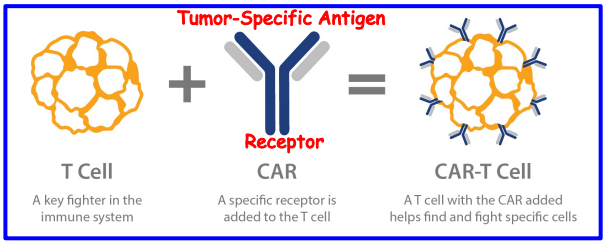
Engineer Killer T-Cells to Find & Destroy Cancer Cells

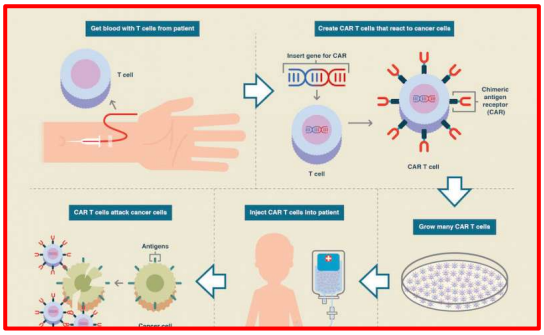
35

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

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

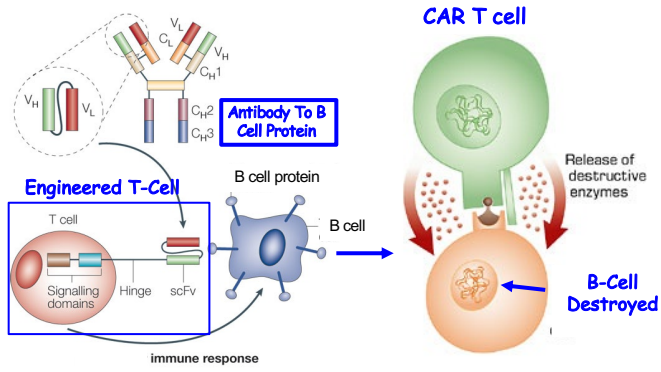
The Antigen is Specific For the Tumor and Binds to Tumor Receptors on Cell Surface





36

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



Protocol

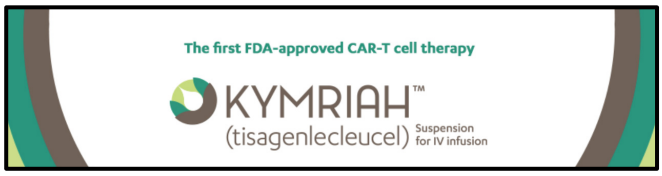
1. Removed T Cells From Patients
2. Engineer Gene Encoding Chimeric Antigen Receptor (CAR) That Recognizes A Protein On The Surface Of B Cells
3. Transferred CAR Gene Into T Cells To Allow Them To Target B Cells
4. Infused CAR T Cells Back Into Patients

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

37

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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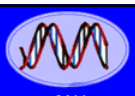

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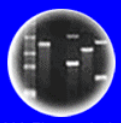

DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

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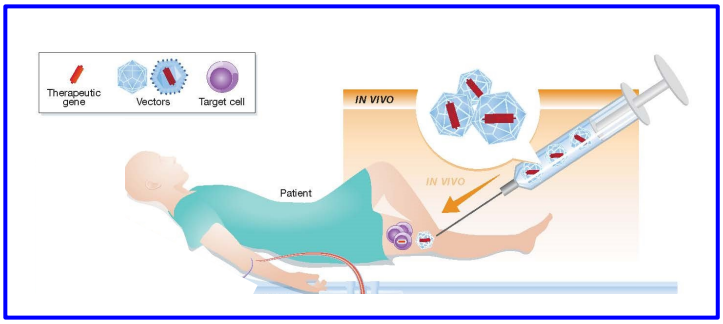
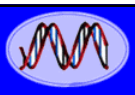
In Vivo Gene Therapy Using Viral Vectors



Replacement of Mutant Recessive Genes

39



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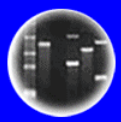

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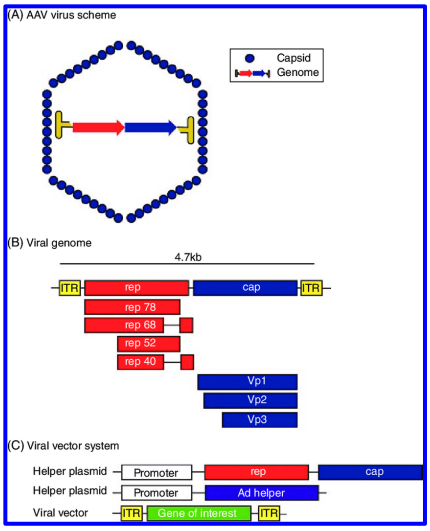
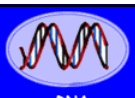
Adeno-Associated (AAV) Viruses Are Viruses that Have a 5kb Single Stranded Genome, Infect a Wide Range of Cell Types, and Are Excellent Vectors For *In Vivo* Gene Therapy



Replacement of Defective Recessive Genes

40



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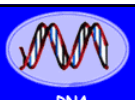
Adeno-Associated Viruses Infect a Wide Range of Cell Types…….

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

41



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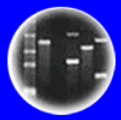

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Cloning: Ethical Issues and Future Consequences

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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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Hemophilia A & B Liver Gene Therapy

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

Factor VIII & IX Deficiency

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

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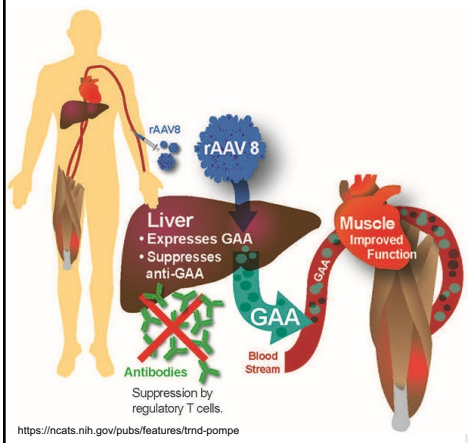
Pompe Disease Liver Gene Therapy



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.

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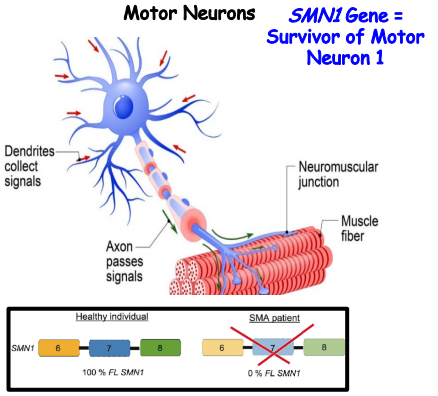
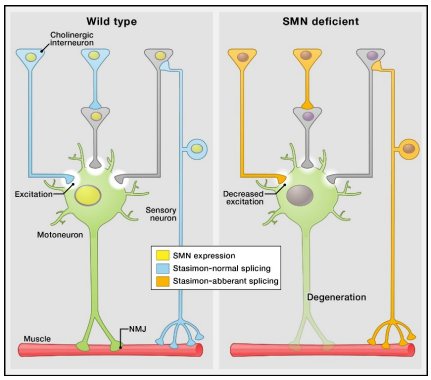
Spinal Muscular Atrophy (SMA) Gene Therapy

- 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

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Spinal Muscular Atrophy Spinal Cord 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


Evelyn Villarreal

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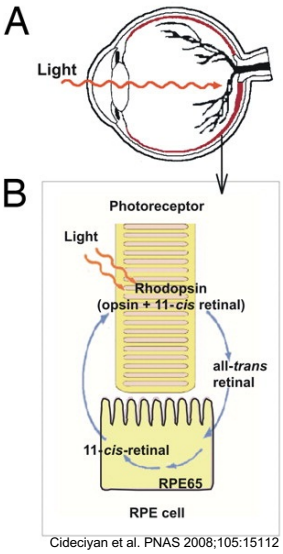
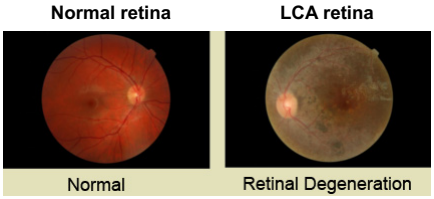
Blindness - *Leber Congenital Amaurosis (LCA)* Gene Therapy

How We See

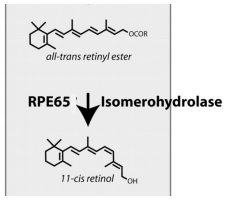


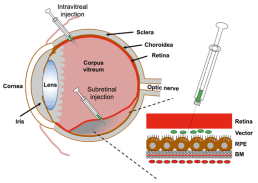
Cideciyan et al. PNAS 2008;105:15112

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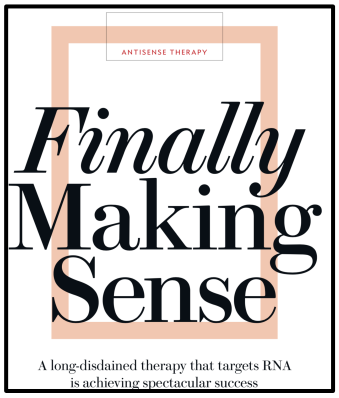
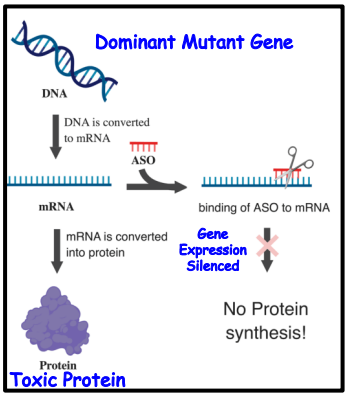
Using ASOs and *In Vivo* Gene Therapy to Silence Gene Activity and Treat Dominant Genetic Disorders





Late Onset Genetic Diseases
Huntington's Disease
Lou Gehrig's Disease - ALS (C9ORF72 Gene)

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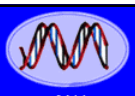


DNA
Genetic Code of Life

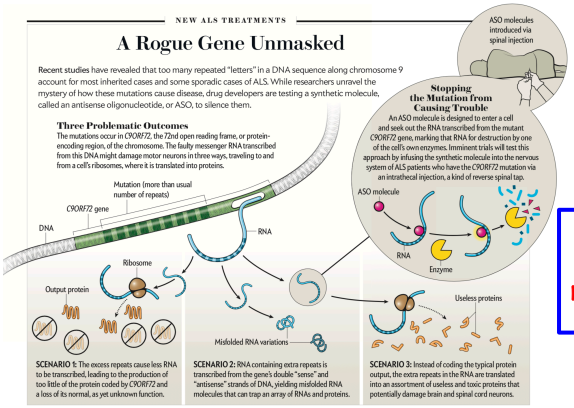
Entire Genetic Code of a Bacteria

DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

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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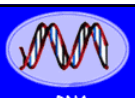

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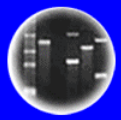

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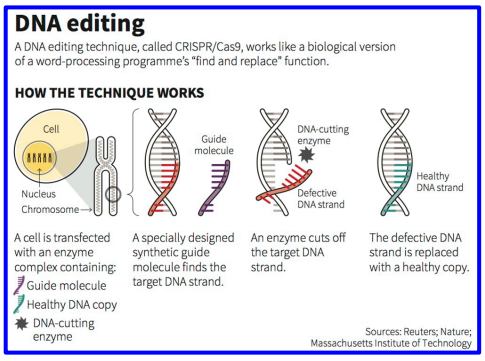
Gene Editing & Human Gene Therapy

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



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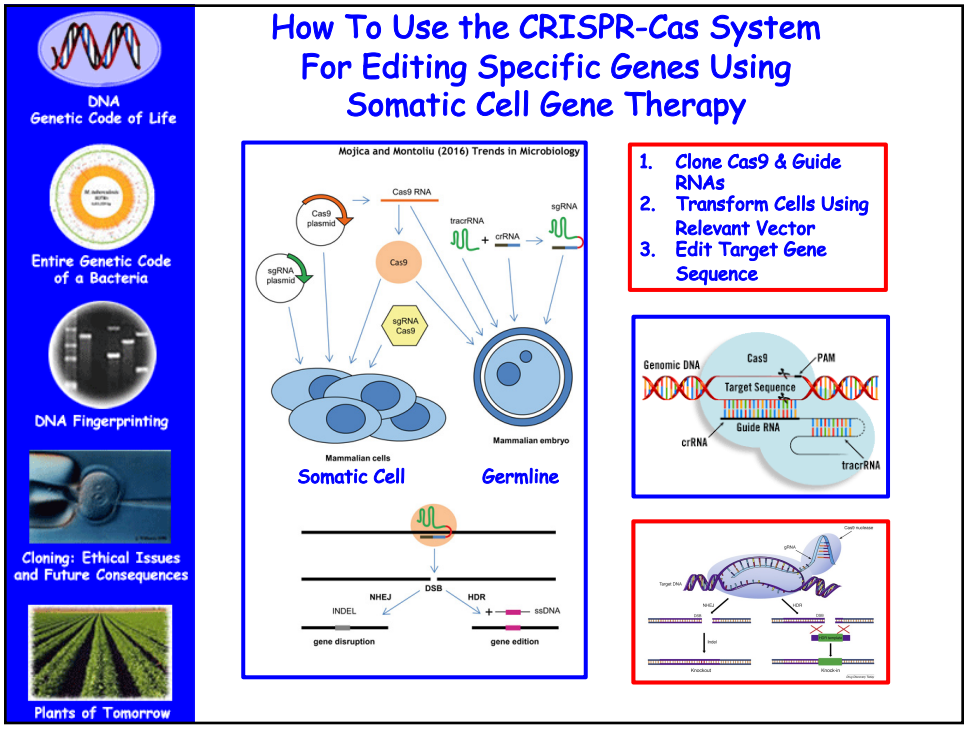
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How To Use the CRISPR-Cas System For Editing Specific Genes Using Somatic Cell Gene Therapy
Mojica and Montoliu (2016) Trends in Microbiology
Cas9 RNA
Cas9 plasmid
sgRNA plasmid
tracrRNA
crRNA
sgRNA
Cas9
sgRNA Cas9
Mammalian cells
Mammalian embryo
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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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
Brian Madeux – First Human Gene Editing Therapy Patient - 2018

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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

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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.

41082 Federal Register / Vol. 83, No. 160 / Friday, August 17, 2018 / Notices

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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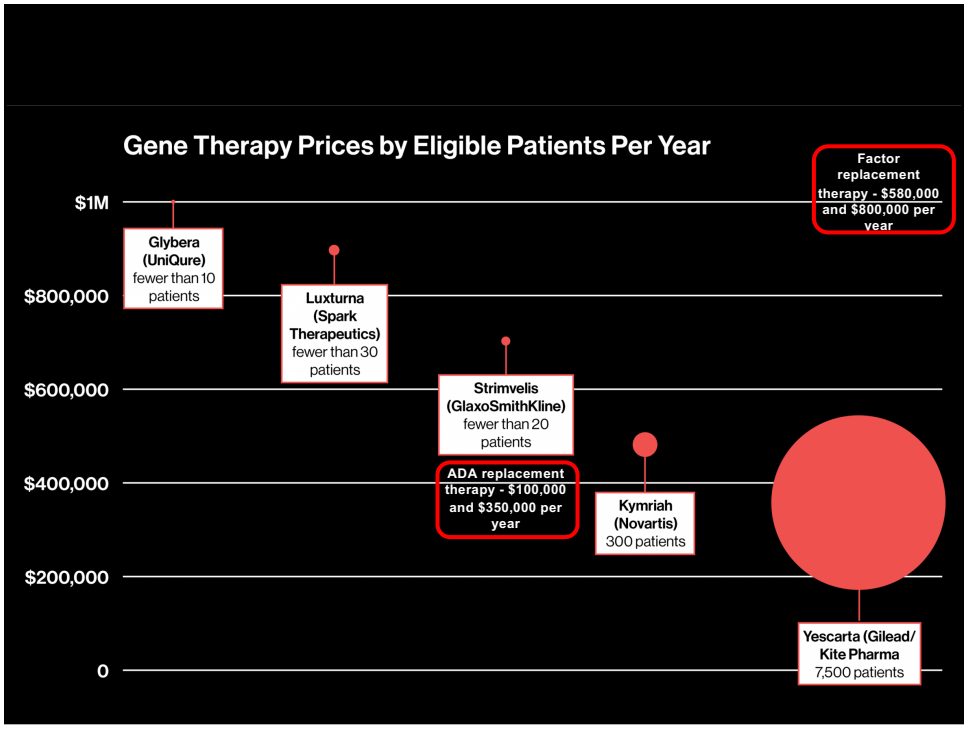
Approved Gene Therapy Products Worldwide
$10B Market By 2024
P53 tumor suppressor deficiency
Marketed in China 2004
SIBIONO
uniQure
Glybera
Lipoprotein lipase deficiency
Marketed in Europe 2012
Strimvelis
ADA-SCID
European Medicines Agency
Approved in 2016
LCA Blindness
FDA Approved 2017
LUXTURNA™
voretigene 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

55


Gene Therapy Prices by Eligible Patients Per Year
$1M
$800,000
$600,000
$400,000
$200,000
0
Glybera (UniQure) fewer than 10 patients
Luxturna (Spark Therapeutics) fewer than 30 patients
Strimvelis (GlaxoSmithKline) fewer than 20 patients
ADA replacement therapy - $100,000 and $350,000 per year
Kymriah (Novartis) 300 patients
Yescarta (Gilead/ Kite Pharma 7,500 patients
Factor replacement therapy - $580,000 and $800,000 per year

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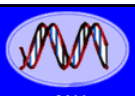

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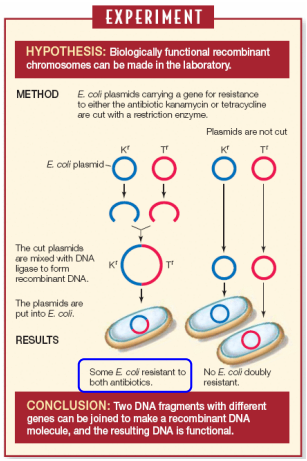
The End!!
Lectures on the History, Science, and Applications of Genetic Engineering……



…….On to Genetic Engineering & the Law

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