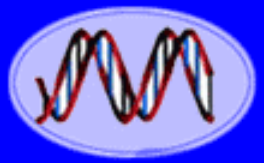
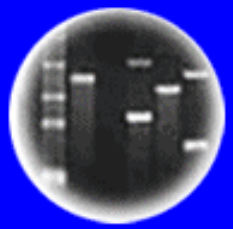


DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

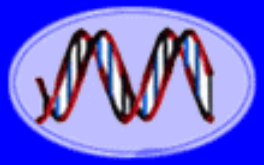
# HC70A & SAS70A Winter 2016
# Genetic Engineering in Medicine, Agriculture, and Law

Professors Bob Goldberg & John Harada

## Lecture 8
## Human Genetic Engineering and Gene Therapy

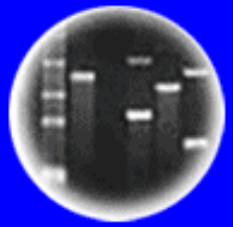
UCLA

UC DAVIS
UNIVERSITY OF CALIFORNIA



DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

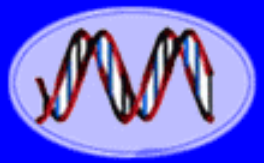
# THEMES

## Human Genetic Engineering and Gene Therapy

1. What is Gene Therapy?
    a. Germ Line
    b. Somatic Cell
2. Two Types of Somatic Cell Gene Therapy
    a. Ex Vivo Gene Therapy
    b. In Vivo Gene Therapy
3. Case Study: Ex Vivo Gene Therapy for Severe Combined Immunodeficiency (SCID)
4. Some Problems and Improvements with Gene Therapy
5. In Vivo Gene Therapy
6. Gene Therapy to Control Cancer
7. Current Status of Gene Therapy
8. Regulations and Issues Concerning Gene Therapy
9. Gene Editing & Human Gene Therapy

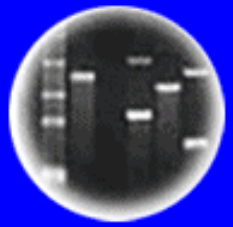
# Genetically Engineered Organisms & Their Uses

1. Bacteria
    a. Drugs
2. Fungi
    a. Drugs
    b. Fermentation
3. Animals
    a. Mouse Model-Knock-Outs-Human Gene Functions
    b. Farm Animals-Drugs
4. Plants
    a. Genetically Engineered Crops
    b. Feedstock for Biofuels



DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



DNA Fingerprinting

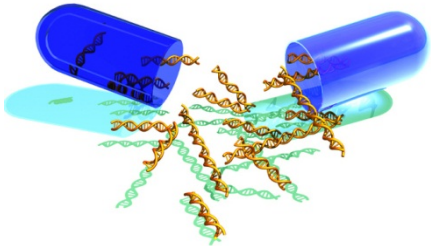
Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

# Human Genetic Engineering and Gene Therapy

# What is Gene Therapy?

- The insertion of usually genetically altered genes into cells especially to replace defective genes in the treatment of genetic disorders or to provide a specialized disease-fighting function - *Merriam-Webster Dictionary*
- Experimental treatment of a genetic disorder by replacing, supplementing, or manipulating the expression of abnormal genes with normally functioning genes - *National Center for Biotechnology*
- It is an approach to treating disease by either modifying the expressions of an individual's genes or correction of abnormal genes - *American Society of Gene and Cell Therapy*
- Gene therapy is the use of DNA as a pharmaceutical agent to treat disease - *Wikipedia*

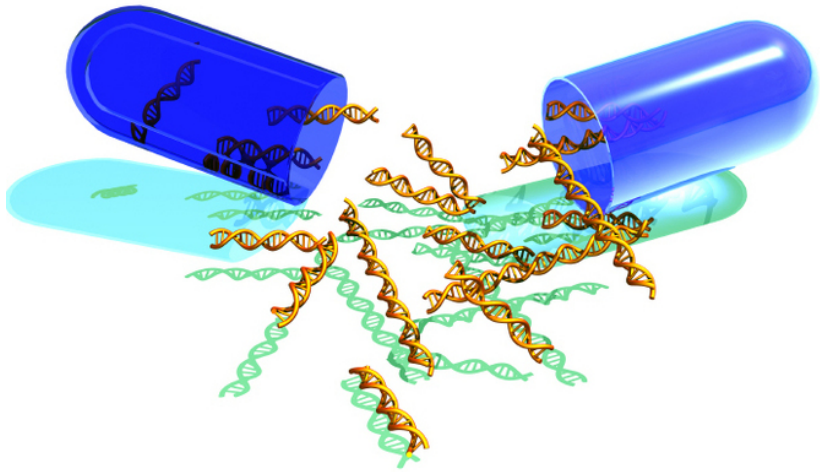


# Types of Gene Therapy

- Germline gene therapy

- Somatic gene therapy
  - Gene supplementation
  - Gene replacement
  - Gene alteration
  - Targeted killing of specific cell-types
  - Targeted silencing of gene expression

- Issues
  - Regulation
  - NIH Guidelines
  - Human Experimentation
  - Ethics
  - Eugenics



## 21.4 Principles of gene therapy

Gene therapy involves the direct genetic modification of cells of the patient in order to achieve a therapeutic goal. There are basic distinctions in the types of cells modified, and the type of modification effected.

① ▶ **Germ-line gene therapy** produces a permanent transmissible modification. This might be achieved by modification of a gamete, a zygote or an early embryo. Germ-line therapy is banned in many countries for ethical reasons (see *Ethics Box 2*).

② ▶ **Somatic cell gene therapy** aims to modify specific cells or tissues of the patient in a way that is confined to that patient. All current gene therapy trials and protocols are for somatic cell therapy.

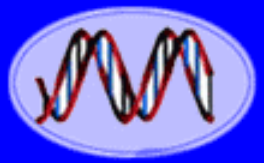
Somatic cells might be modified in a number of different ways (*Figure 21.4*).

a. ▶ **Gene supplementation** (also called gene augmentation) aims to supply a functioning copy of a defective gene. This would be used to treat loss-of-function conditions (Section 16.4) where the disease process is the result of a gene not functioning here and now. Cystic fibrosis would be a typical candidate. It would not be suitable for loss-of-function conditions where irreversible damage has already been done, for example through some failure in embryonic development. Cancer therapy could involve gene supplementation to increase the immune response against a tumor or to replace a defective tumor suppressor gene.

b. ▶ **Gene replacement** is more ambitious: the aim is to replace a mutant gene by a correctly functioning copy, or to correct a mutation *in situ*. Gene replacement would be required for gain-of-function diseases where the resident mutant gene is doing something positively bad.

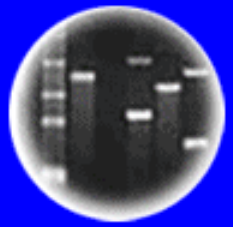
c. ▶ **Targeted inhibition of gene expression** is especially relevant in infectious disease, where essential functions of the pathogen are targeted. It could also be used to silence activated oncogenes in cancer, to damp down unwanted responses in autoimmune disease and maybe to silence a gain-of-function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

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



DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



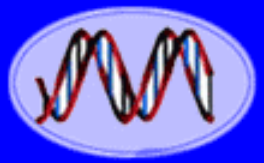
DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

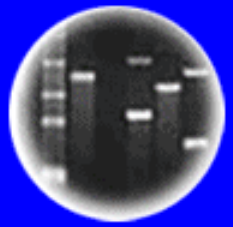
Which type(s) of gene therapy should be allowed?

a. Germline cell gene therapy
b. Somatic cell gene therapy
c. Both
d. Neither



DNA
Genetic Code of Life

Entire Genetic Code of a Bacteria



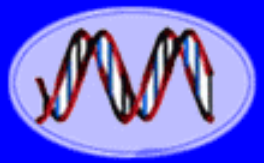
DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

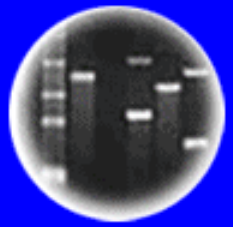
It is illegal to conduct the following type(s) of gene therapy in the United States.

a. Germline cell gene therapy
b. Somatic cell gene therapy
c. Both
d. Neither



DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

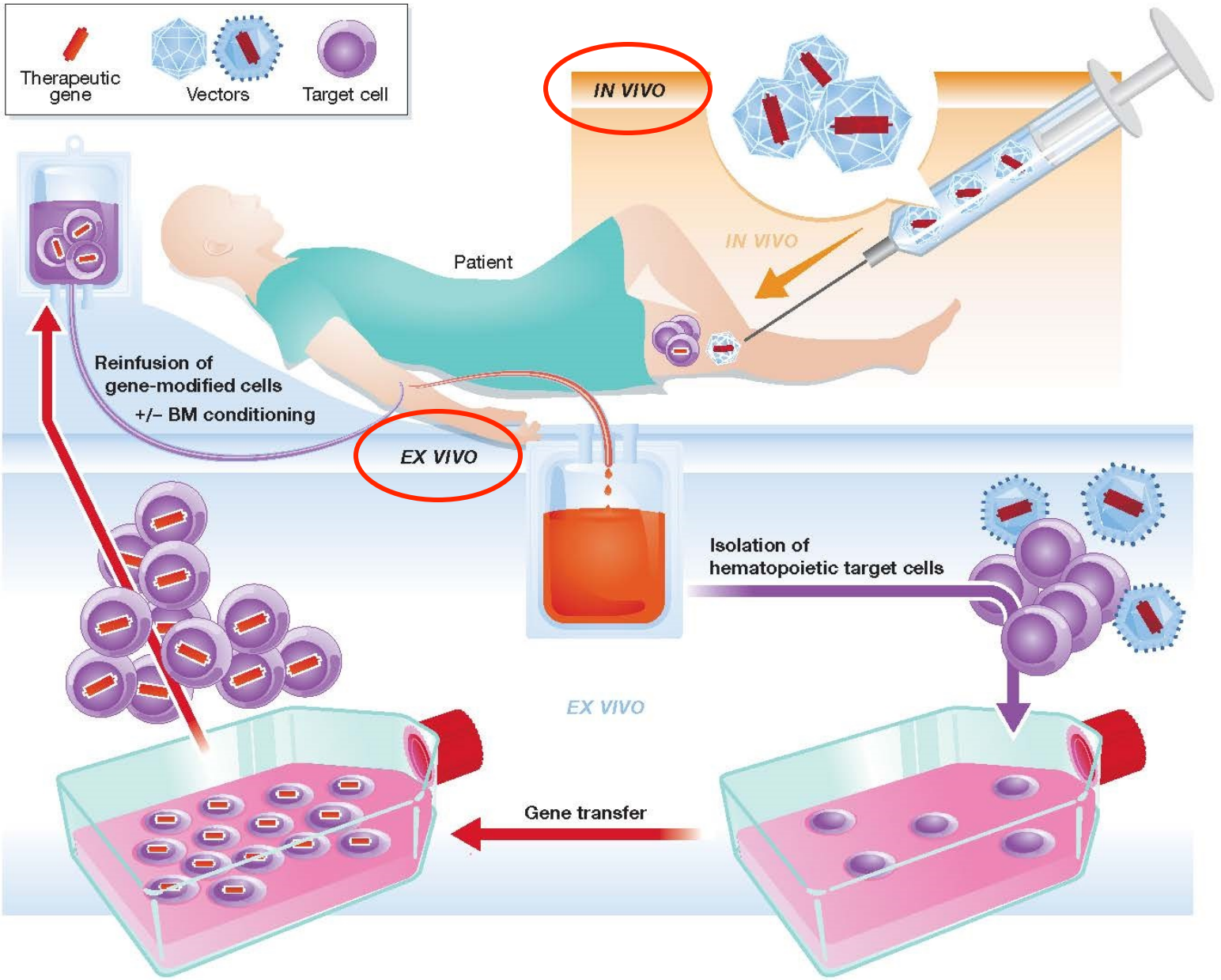
How many approved gene therapy "products" are available in the United States?

a. More than 100
b. 11 to 100
c. 1 to 10
d. 0

# Questions to Consider Before Initiating Gene Therapy

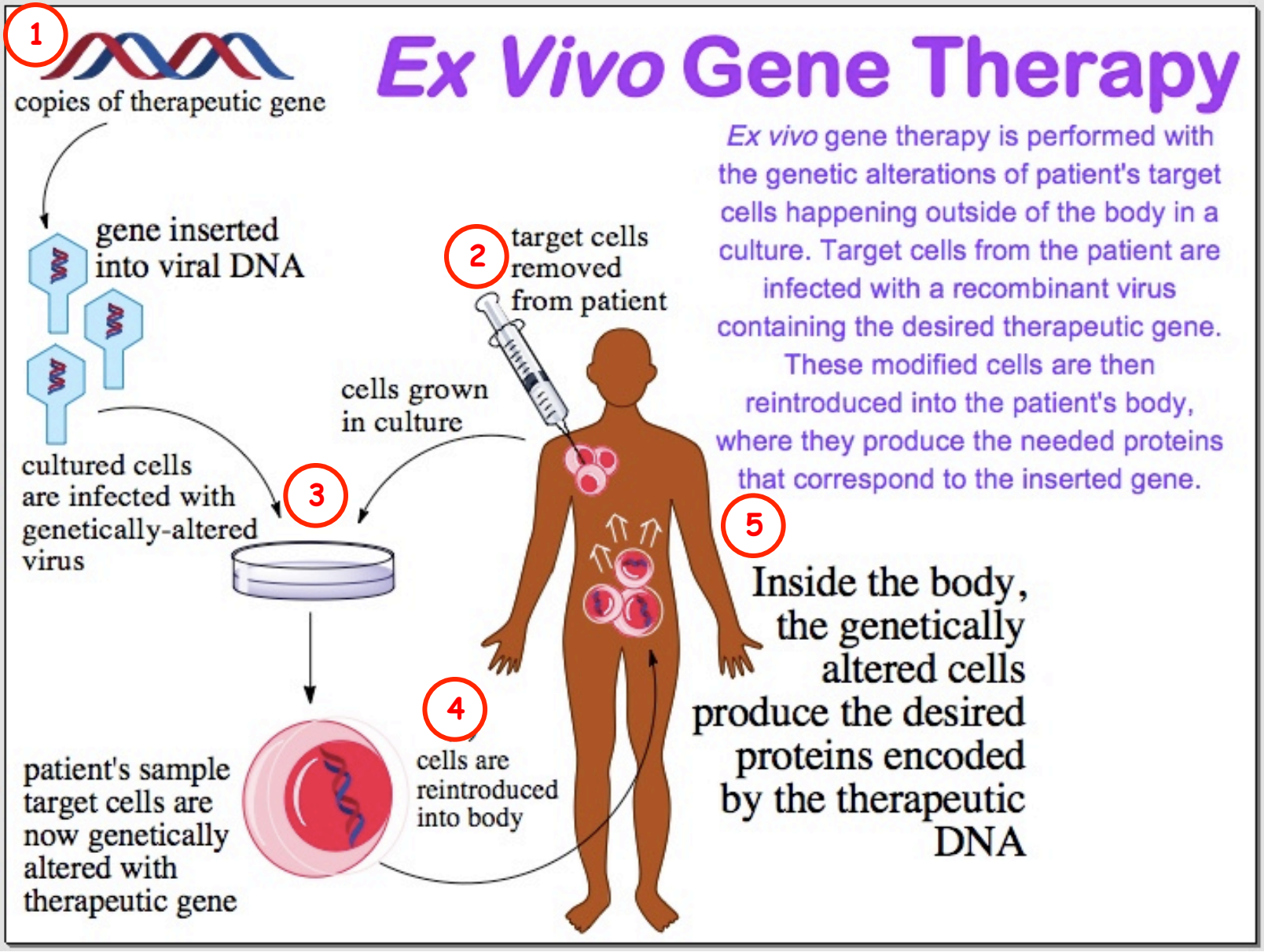
1. What is known about the biology of the disorder?
2. Does the condition result from a mutation of one or more genes?
3. Has the affected gene been cloned?
4. Will adding a normal copy of the gene fix the problem in the affected tissue?
5. Can you deliver the gene to cells of the affected tissue?

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



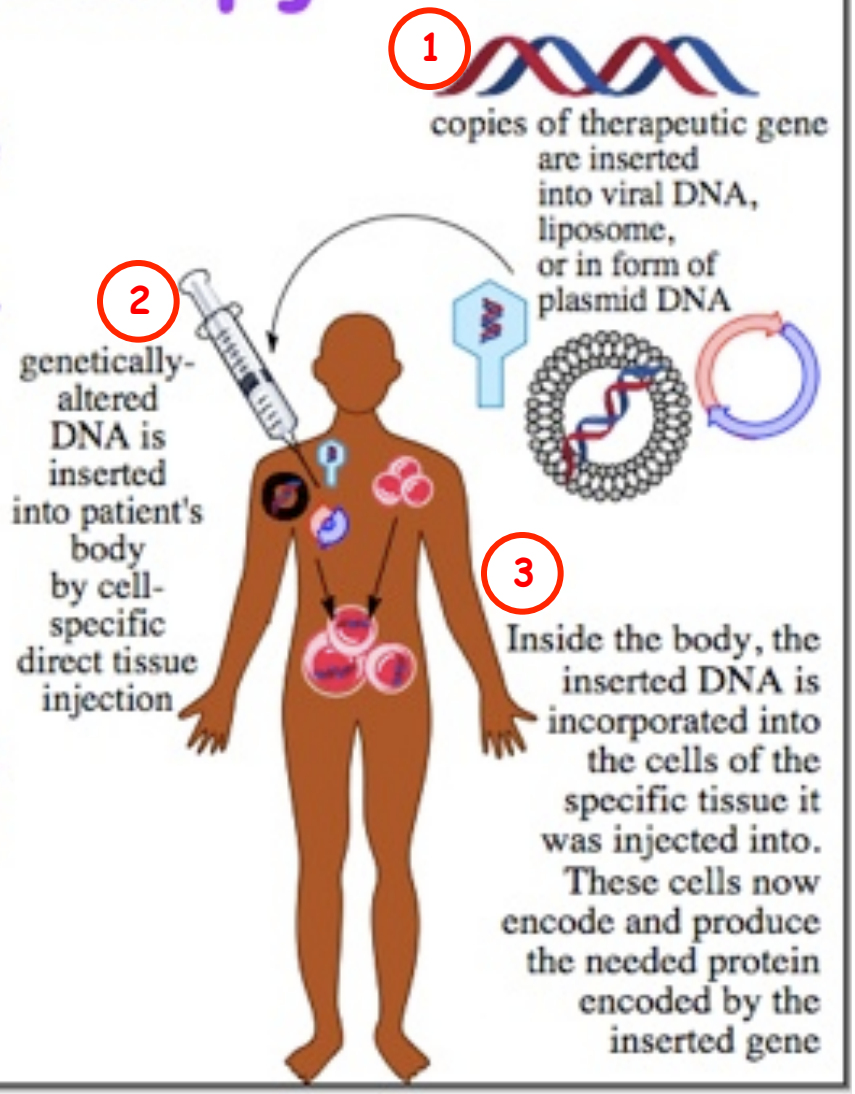
# *Ex Vivo* Gene Therapy

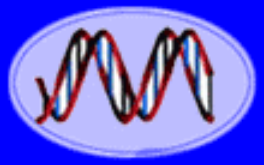
*Ex vivo* gene therapy is performed with the genetic alterations of patient's target cells happening outside of the body in a culture. Target cells from the patient are infected with a recombinant virus containing the desired therapeutic gene. These modified cells are then reintroduced into the patient's body, where they produce the needed proteins that correspond to the inserted gene.



# *In Vivo* Gene Therapy

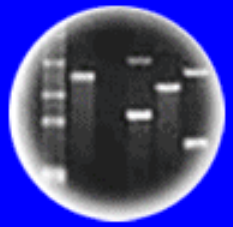
In vivo gene therapy involves introduction of therapeutic DNA directly into the patient's body. The DNA is introduced by cell-specific direct injection into tissue in need. DNA in the form of a plasmid vector is introduced by a dermal vaccination. Modified liposomes are not currently used for gene therapy, but they will likely be the next advancement in therapeutic gene delivery as cell-specific receptor-mediated DNA carriers. Once inside the body and in contact with the specifically targeted cells, the inserted DNA is incorporated into the tissue's cells where it encodes the production of the needed protein.





DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



DNA Fingerprinting

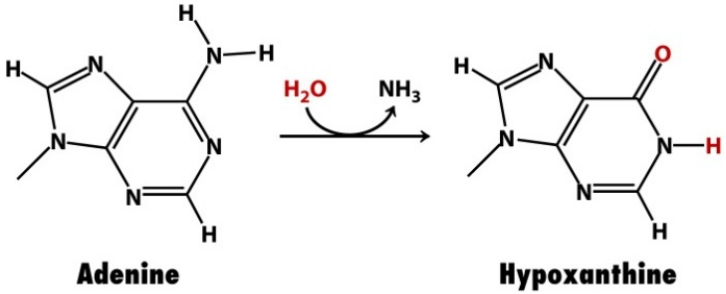
Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

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

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

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

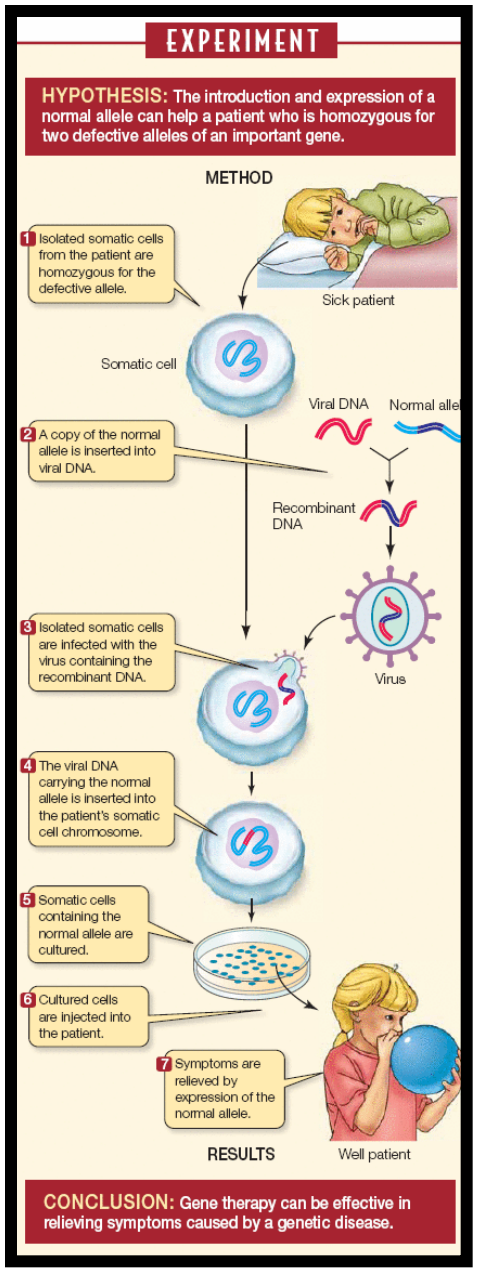



Degradation of Purine

David Vetter - Died at Age 12

- ADA is an enzyme that metabolizes adenosine and deoxyadenosine
- ADA deficiency results in elevated adenosine and deoxyadenosine levels
- Abnormal levels impair lymphocyte development and function
- **The immune system is severely compromised or completely defective**
- ADA deficiency accounts for ~15% of all SCID cases
- ADA-SCID patients can be treated with PEG-ADA, a stabilized form of the enzyme

# Humans Have Been Genetically Engineered To Cure a Lethal Genetic Disease (SCID)




## Gene therapy cures 'bubble boy disease'

31 Jan 2009, 1128 hrs IST, AP

**The Age of Human Genetic Engineering Began More than Twenty Years Ago – SCID Treated with a Normal ADA Gene!!!**

**Several People are Alive Because They Have Been Engineered with an ADA Gene**

Ashanthi DeSilva

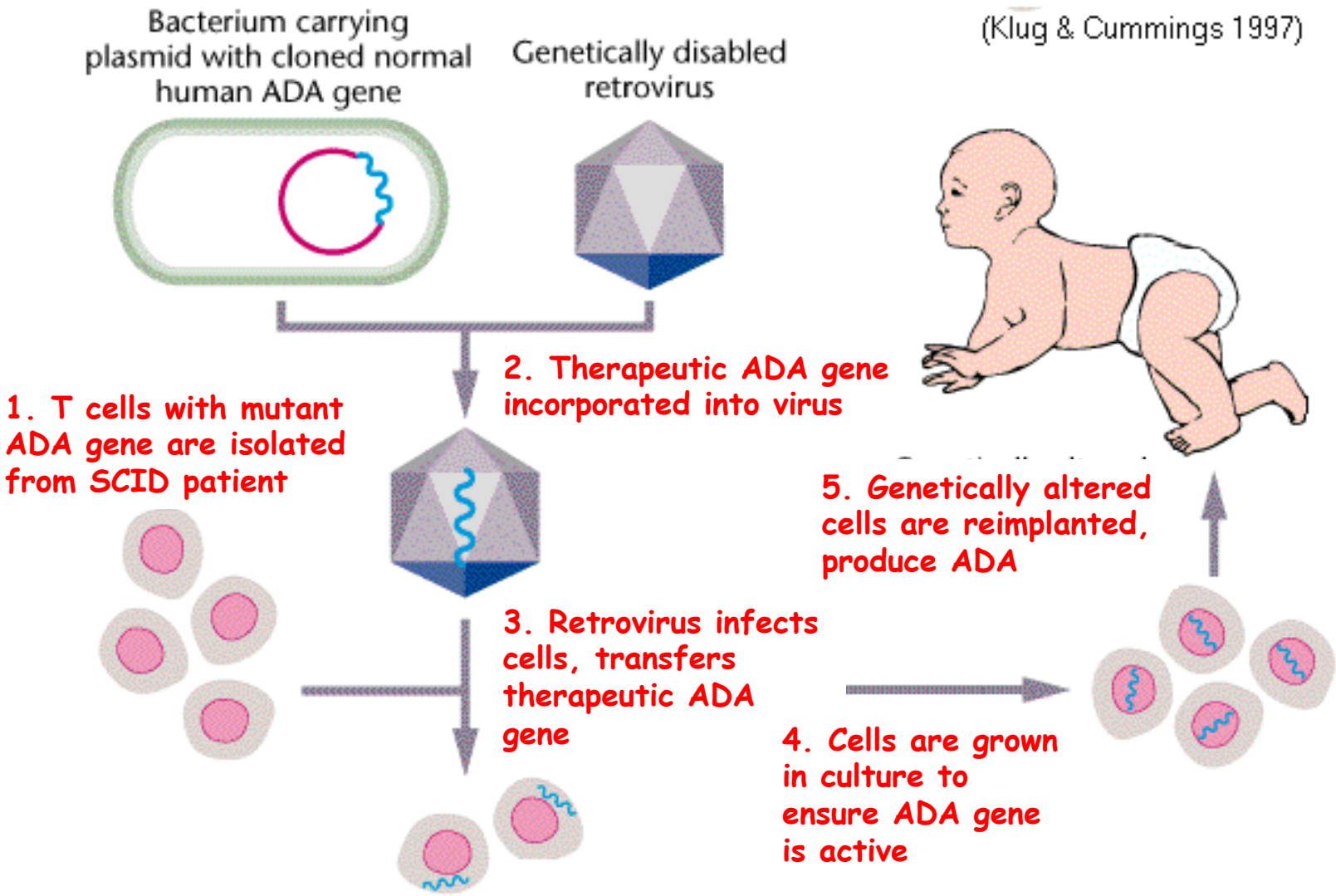
The new england journal of medicine

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

Gene Therapy for Immunodeficiency Due to Adenosine Deaminase Deficiency

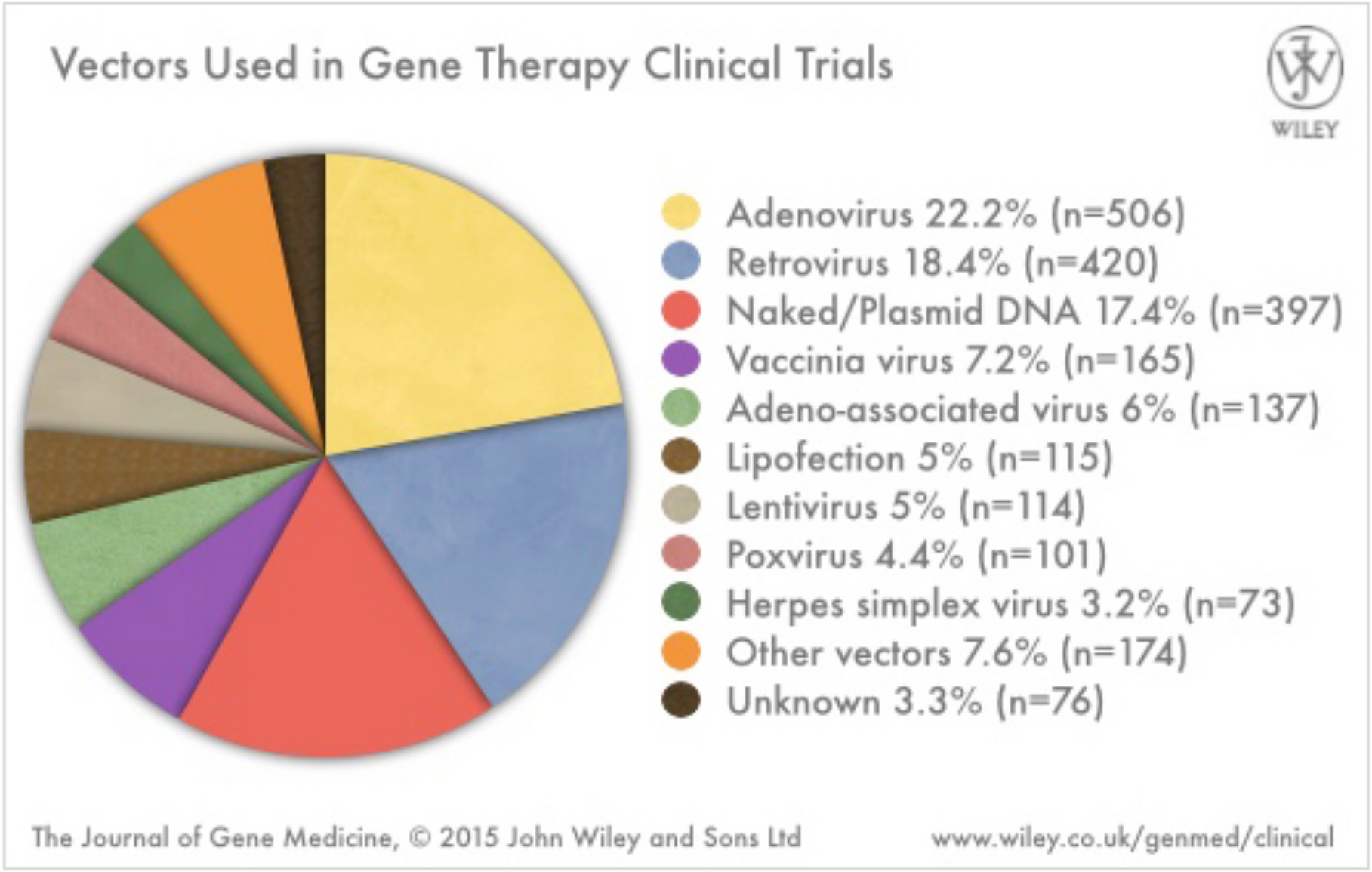
***Gene Therapy with the Adenosine Deaminase (ADA) Gene***

# Ex Vivo Gene Therapy for Severe Combined Immunodeficiency (SCID)




## ADA-SCID Clinical Trial Started in 1990

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



# Vectors Used to Deliver Genes to Cells in Gene Therapy

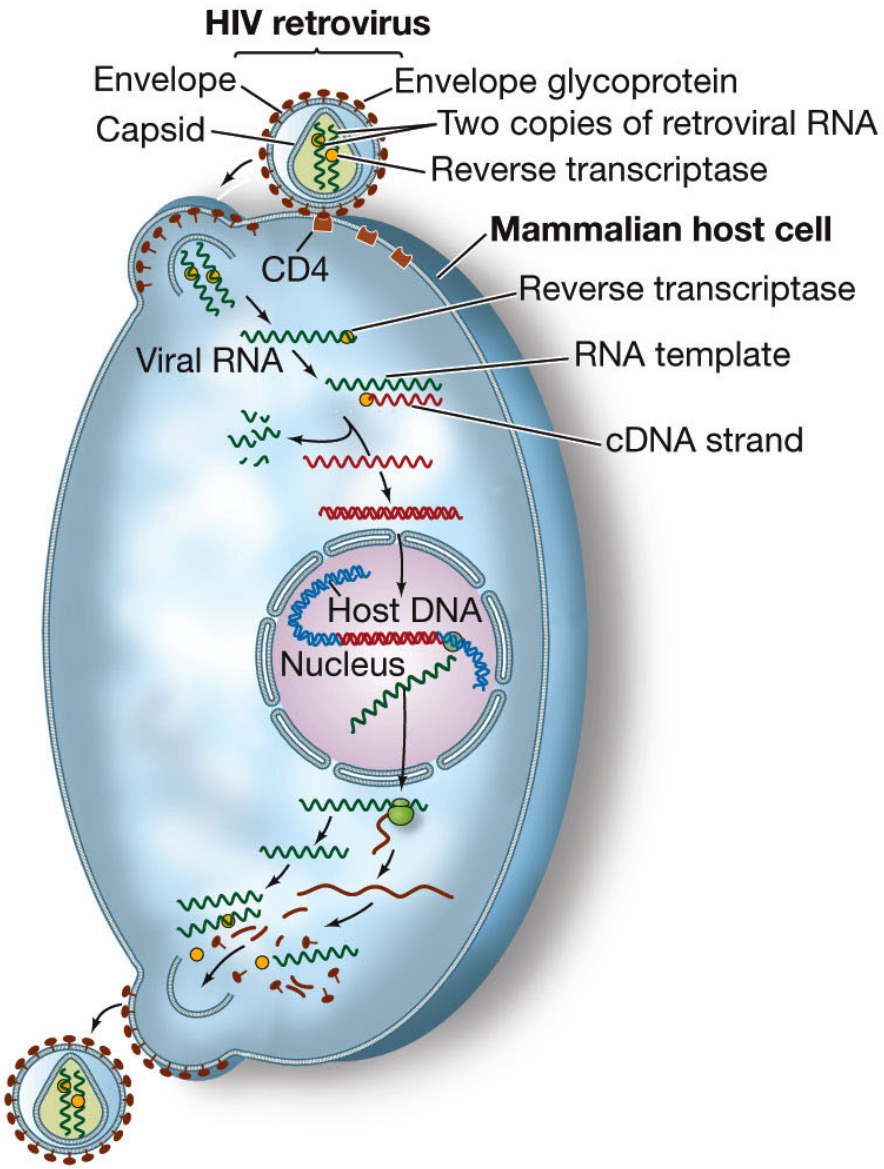
**Table 19.3 Vectors used in gene therapy**

| Vector | Advantages | Disadvantages |
|---|---|---|
| Retrovirus | Efficient transfer | Transfers DNA only to dividing cells, inserts randomly; risk of producing wild-type viruses |
| Adenovirus | Transfers to nondividing cells | Causes immune reaction |
| Adeno-associated virus | Does not cause immune reaction | Holds small amount of DNA; hard to produce |
| Herpes virus | Can insert into cells of nervous system; does not cause immune reaction | Hard to produce in large quantities |
| Lentivirus | Can accommodate large genes | Safety concerns |
| Liposomes and other lipid-coated vectors | No replication; does not stimulate immune reaction | Low efficiency |
| Direct injection | No replication; directed toward specific tissues | Low efficiency; does not work well within some tissues |
| Pressure treatment | Safe, because tissues are treated outside the body and then transplanted into the patient | Most efficient with small DNA molecules |
| Gene gun (DNA coated on small gold particles and shot into tissue) | No vector required | Low efficiency |

Source: After E. Marshall, Gene therapy's growing pains, *Science* 269:1050–1055, 1995.

Table 19-3
*Genetics: A Conceptual Approach, Third Edition*

# HIV is a Retrovirus




T-Cell

*LIFE 8e*, Figure 13.6

# Discovery of Retroviruses

The Retrovirus Genome Encodes Reverse Transcriptase

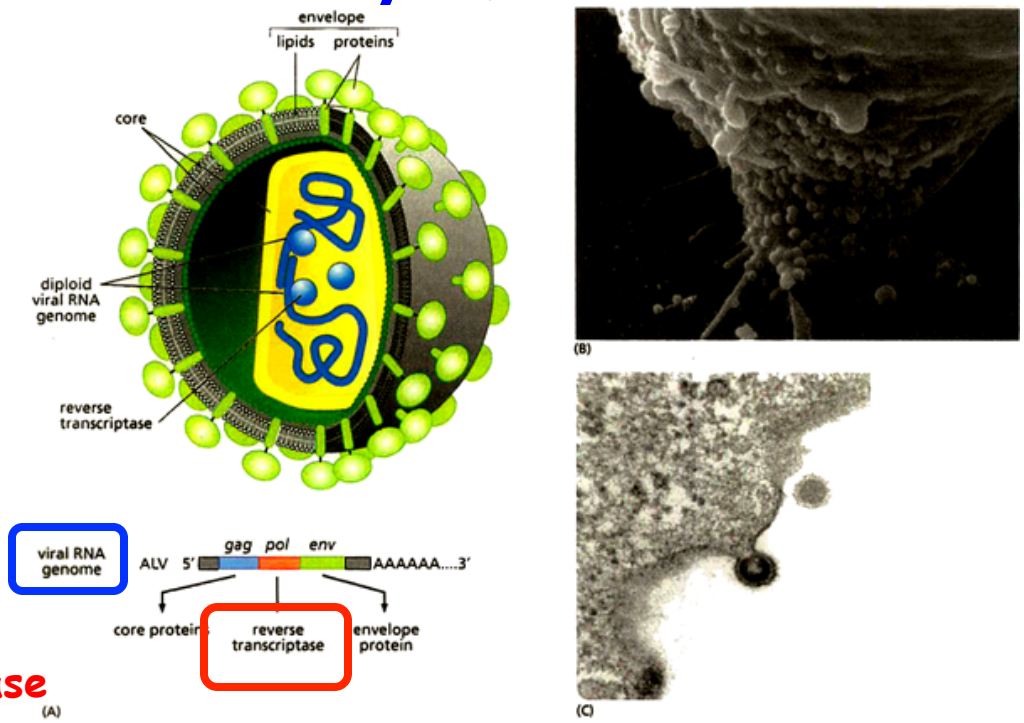



**Figure 3.4 The virion of RSV and related viruses** (A) This schematic drawing of the structure of a *retrovirus* virion, such as that of Rous sarcoma virus, indicates three major types of viral proteins. The glycoprotein spikes (encoded by the viral *env* gene) protrude from the lipid bilayer that surrounds the virion; these spikes enable the virion to *adsorb* (attach) to the surface of a cell and to introduce the internal contents of the virion into its cytoplasm. These include a complex protein coat formed by the several core proteins encoded by the viral *gag* gene. Within this protein shell are found two identical copies of the viral genomic the viral *pol* gene. (B) Scanning electron micrograph and (C) transmission electron micrograph showing murine leukemia virus (MLV) particles budding from the surface of an infected cell. As the nucleocapsids (containing the gag proteins, the virion RNA, and the reverse transcriptase) leave the cell, they wrap themselves with a patch of lipid bilayer taken from the plasma membrane of the infected cell. (A, adapted from H. Fan et al., The Biology of AIDS. Boston, MA: Jones and Bartlett Publishers, 1989; B, courtesy of Albert Einstein College of Medicine; C, courtesy of Laboratoire de Biologie Moleculaire.)

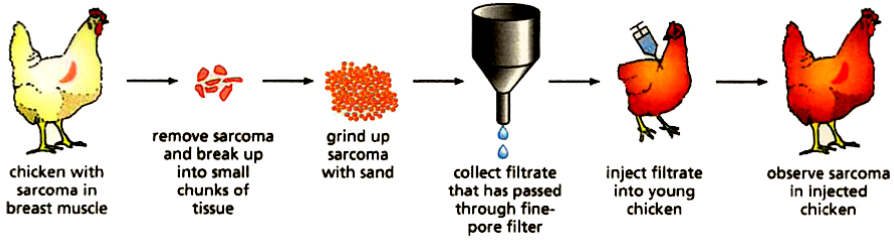
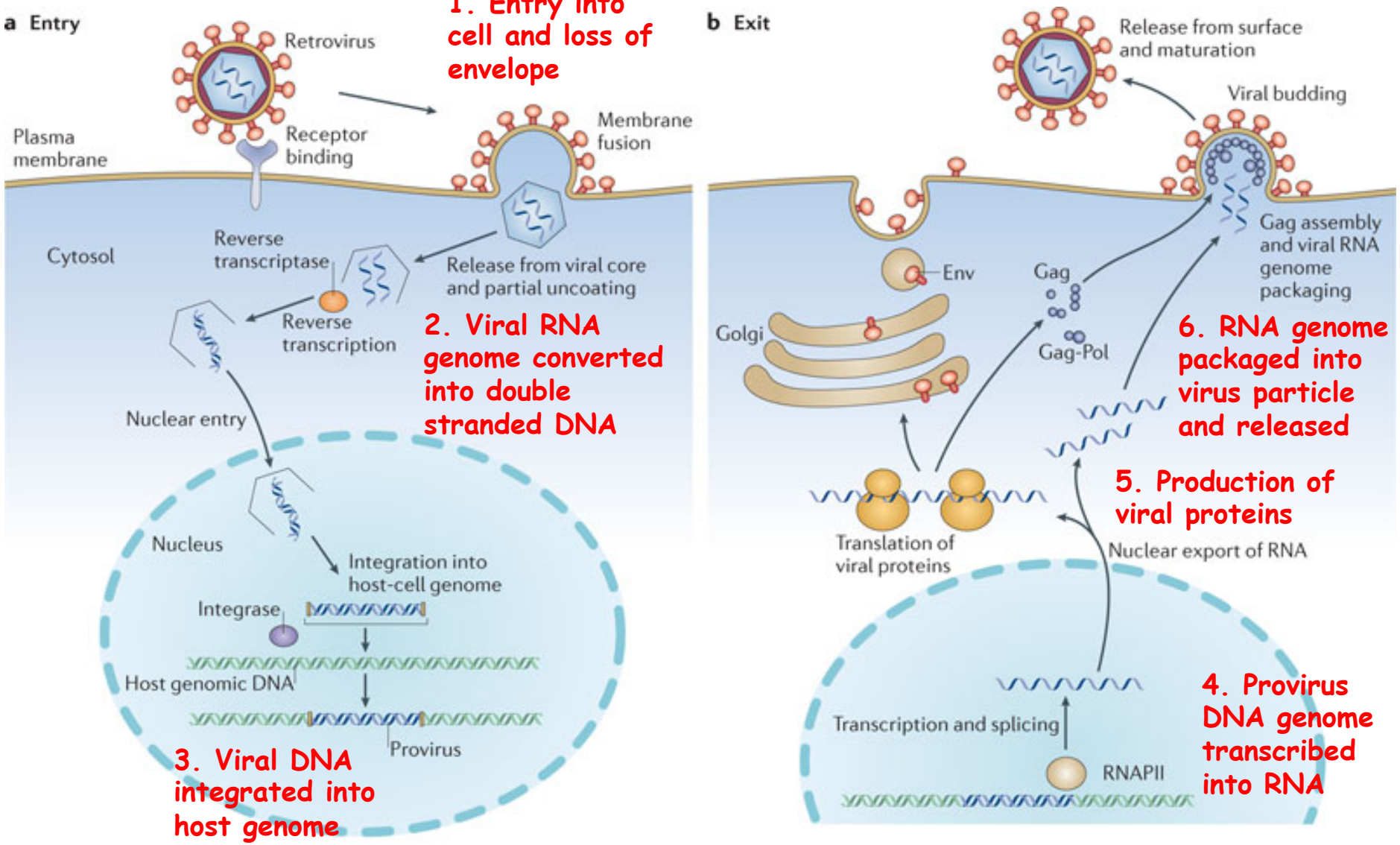



**Figure 3.2 Rous's protocol for inducing sarcomas in chickens** Rous removed a sarcoma from the breast muscle of a chicken, ground it with sand, and passed the resulting homogenate through a fine-pore filter. He then injected the filtrate (the liquid that passed through the filter) into the wing web of a young chicken and observed the development of a sarcoma many weeks later. He then ground up this new sarcoma and repeated the cycle of homogenization, filtration, and injection, once again observing a tumor in another young chicken. These cycles could be repeated indefinitely; after repeated serial passaging, the virus was able to produce sarcomas far more rapidly than the original viral isolate.

Rous Sarcoma Virus is a Retrovirus That Causes Cancer and Contains Oncogenes in its Genome

Francis Peyton Rous
Nobel Prize, 1966

# Retrovirus Life Cycle



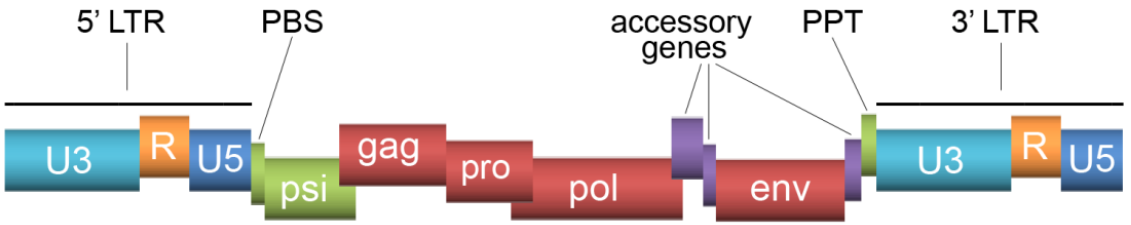

Retroviruses Replicate Using Reverse Transcriptase

David Baltimore & Howard Temin-Nobel Prize 1975

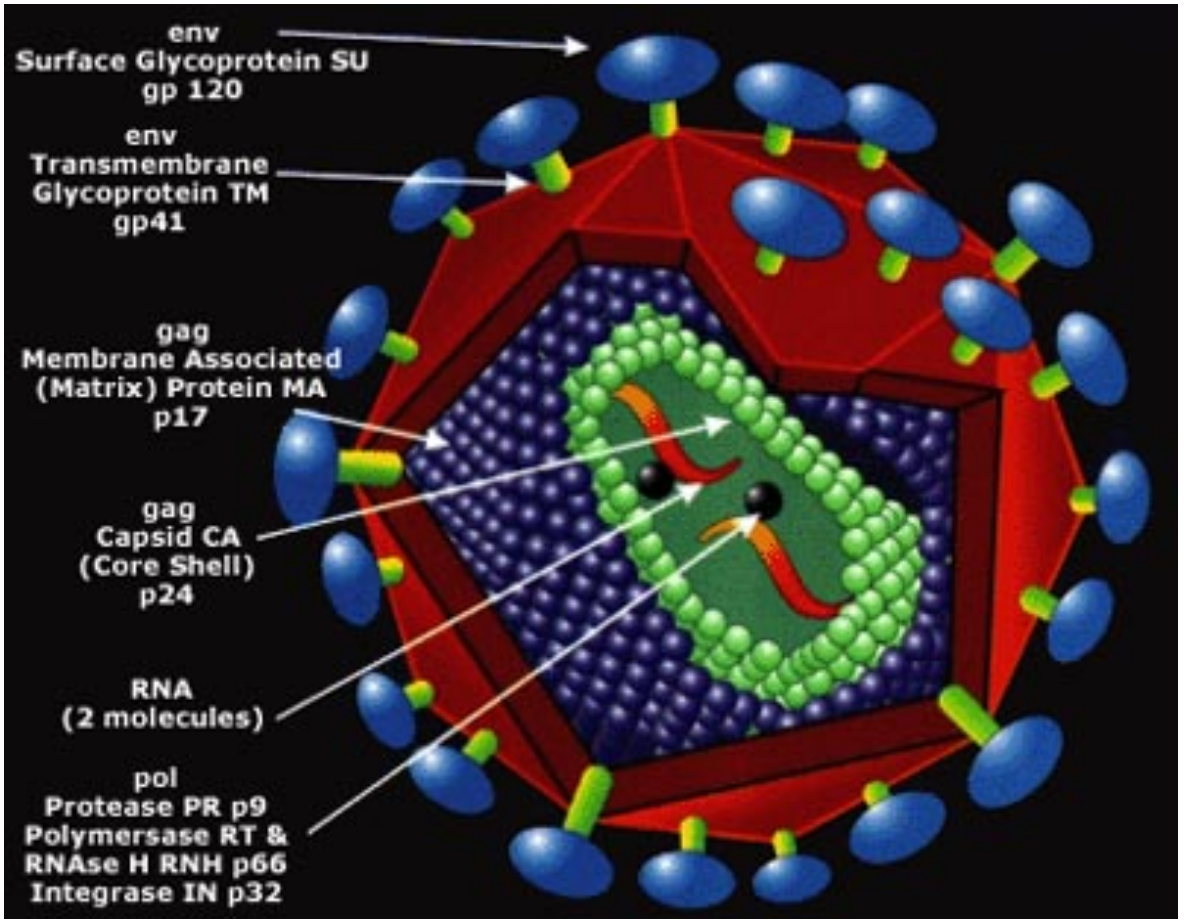
**Modification of the Central Dogma of Molecular Biology**

Nature Reviews | Microbiology

# Retrovirus Genome

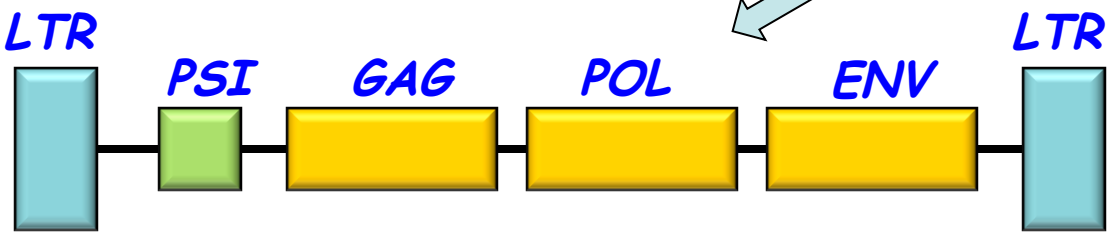


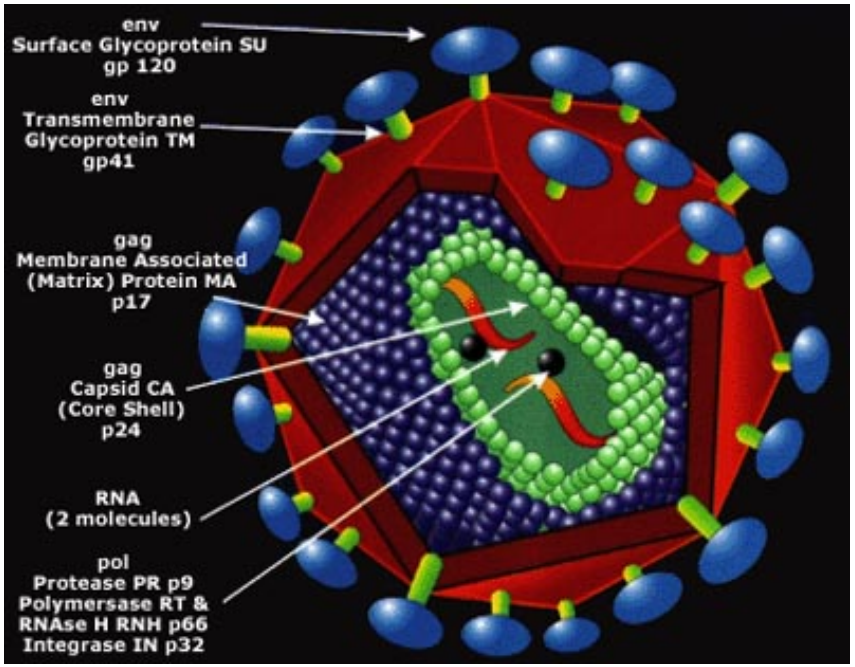

- 5' long terminal repeat (LTR) – switch
- 3' LTR – transcriptional termination
- psi – packaging element needed to package the RNA genome into the viral particle
- gag – structural (coat) proteins
- pro – protease
- pol – reverse transcriptase
- env – envelope proteins



# Retroviral Vector

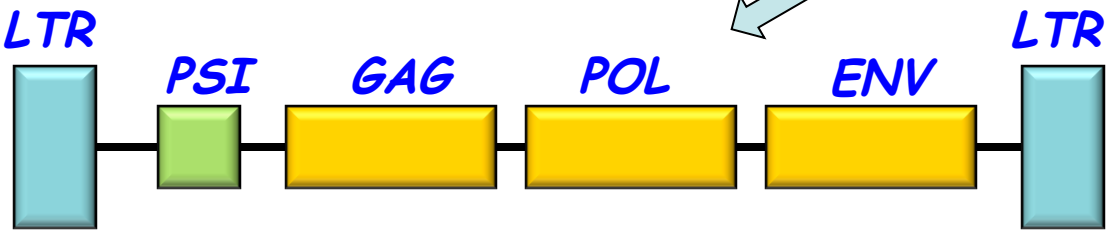

Retrovirus



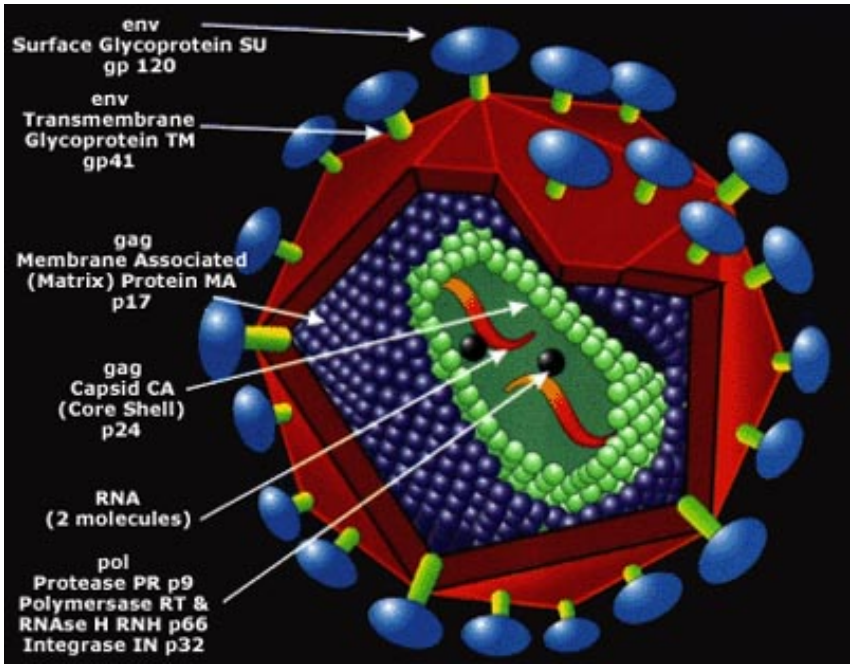



# Retroviral Vector

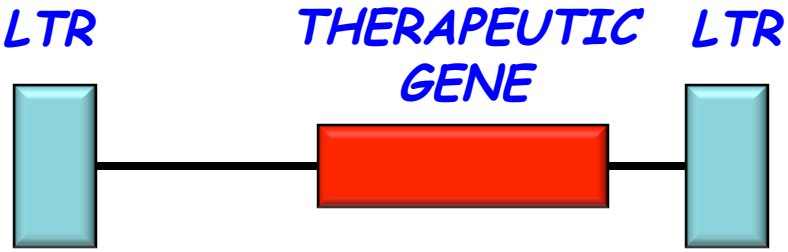
THERAPEUTIC GENE




## Retrovirus



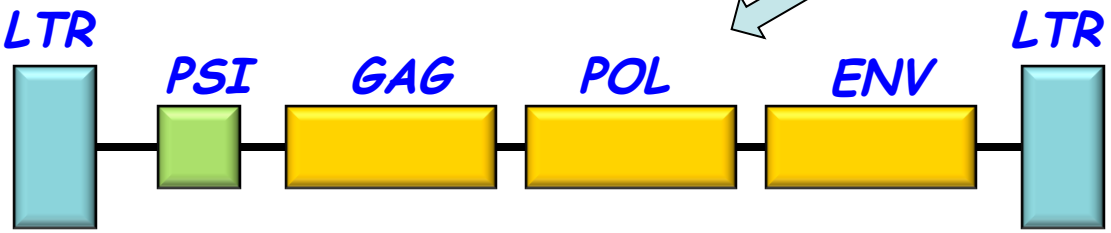

## Retroviral Gene Therapy Vector

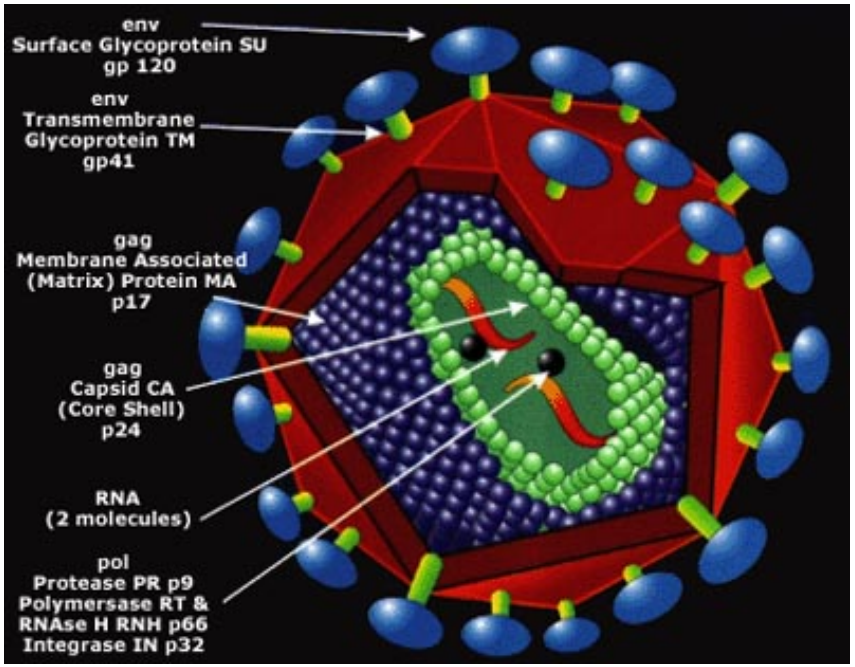


# Retroviral Vector

THERAPEUTIC GENE

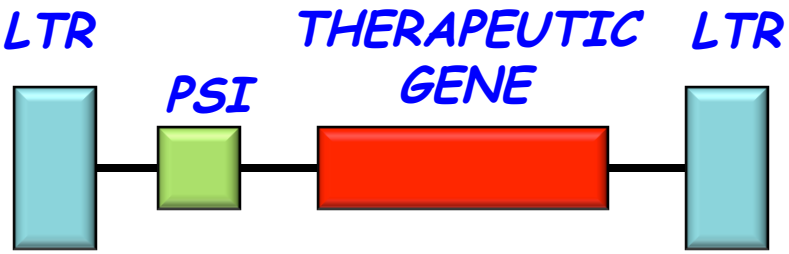
Retrovirus



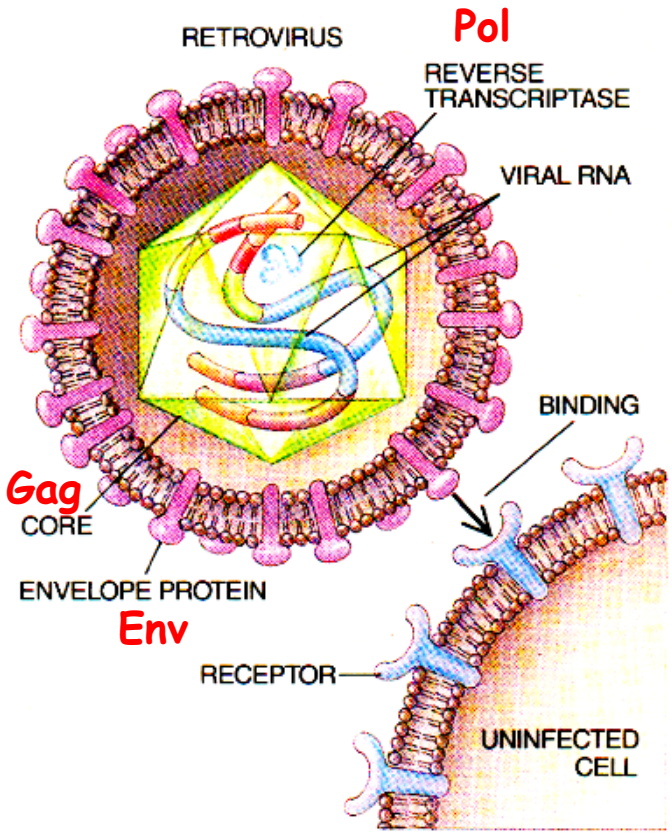



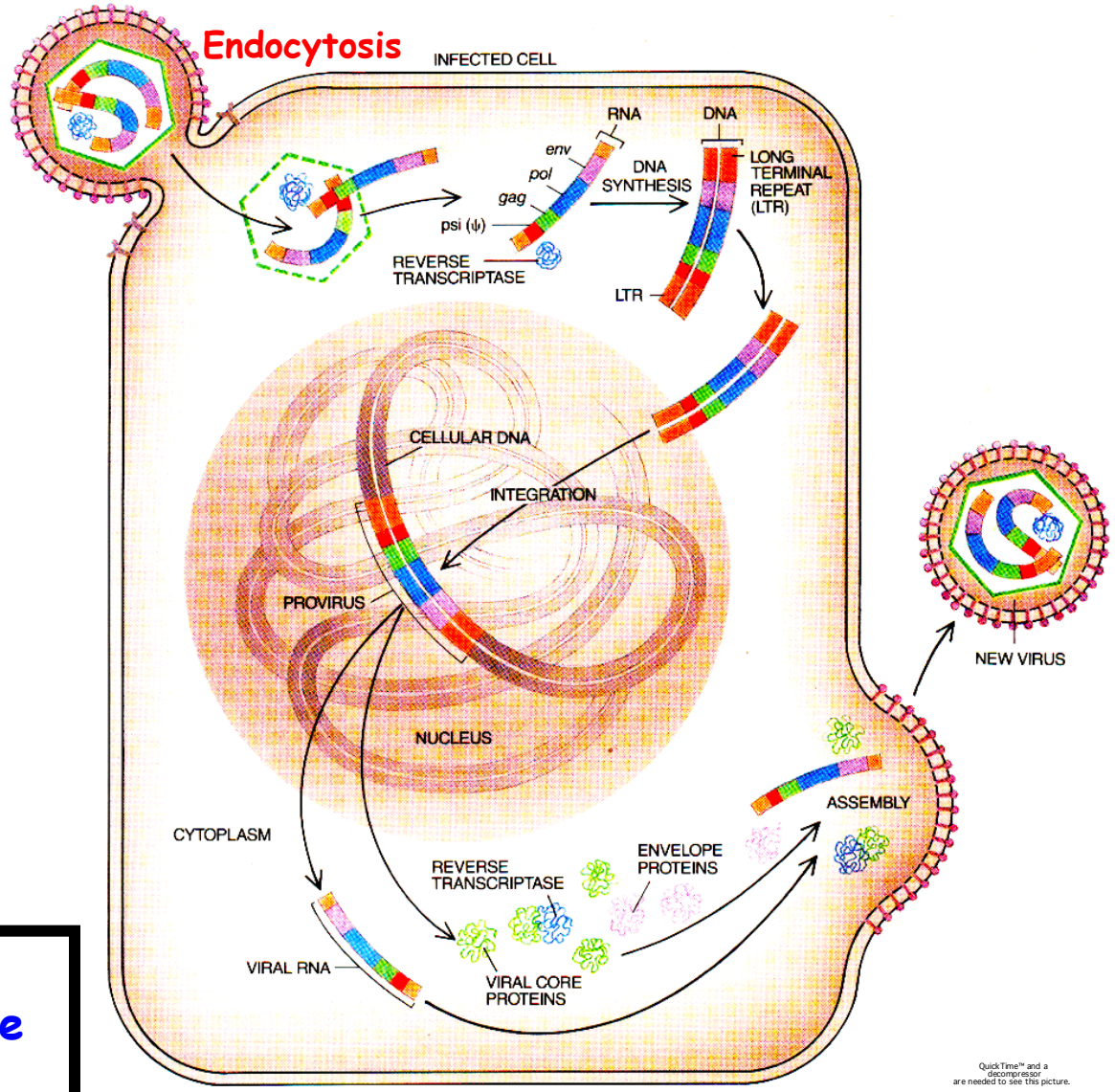

*PSI* is a packaging element – a RNA sequence that is required for the viral RNA to be packaged into the virus

## Retroviral Gene Therapy Vector



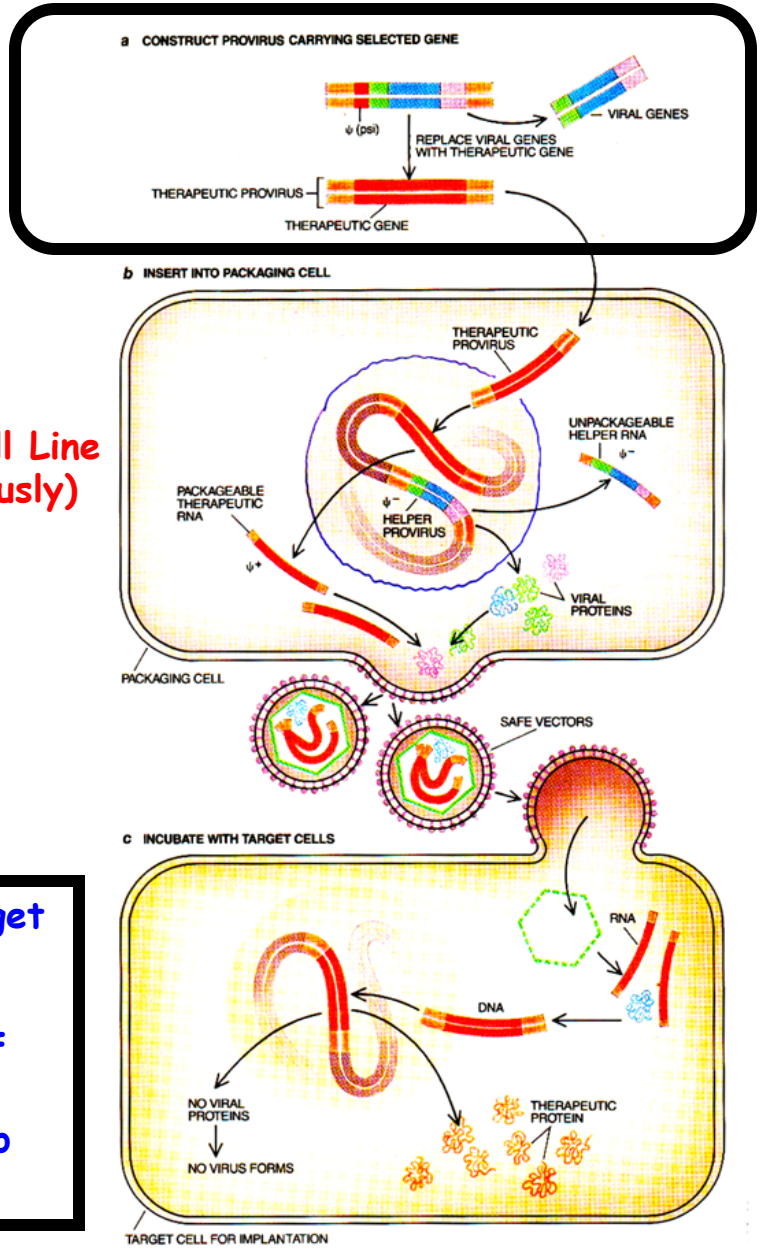
# Using a Retrovirus as a Vector For Human Ex Vivo Gene Therapy




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

# Using Retroviruses for Ex Vivo Gene Therapy




**A.**

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

**B.**

1. Packaging Cells Makes Viral Proteins
2. Cannot Package (Ψ-Minus)
3. Packages Therapeutic Transcript (Ψ-Plus)

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

# Setbacks for Gene Therapy

The New York Times 1999

## The Biotech Death of Jesse Gelsinger

By Sheryl Gay Stolberg
Published: November 28, 1999

- Gelsinger had a mild form of ornithine transcarbamylase (OTC) deficiency – results in an inability to metabolize ammonia
- He volunteered for clinical trial of gene supplementation therapy and was injected with adenovirus vector containing OTC gene
- He died of systemic inflammatory response syndrome – immune reaction to adenovirus vector

The New York Times 2002

## TRIALS ARE HALTED ON A GENE THERAPY

By SHERYL GAY STOLBERG
Published: October 4, 2002

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

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

# Some Early Problems with Human Gene Therapy

- Inefficient delivery of vector to target cells
- Low expression level of therapeutic gene
- Adverse immune reactions to vector
- Insertional mutagenesis causing other diseases (e.g., leukemia)
- Human error - failure to adhere to strict NIH and IRB procedures (experimental therapies)

# A Comeback for Gene Therapy

Science
AAAS

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

**Forbes** / Pharma & Healthcare

The Little Black Book of Billionaire Secrets

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

## Gene Therapy's Big Comeback

**Article**

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

Maria P Limberis

HEALTH

## Gene Therapy Makes a Comeback With a Cautious, Supporting Role

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

BloombergBusiness

## Money Flows Again to Gene-Therapy Drugs Investors Once Shunned

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

**Yang Lu**

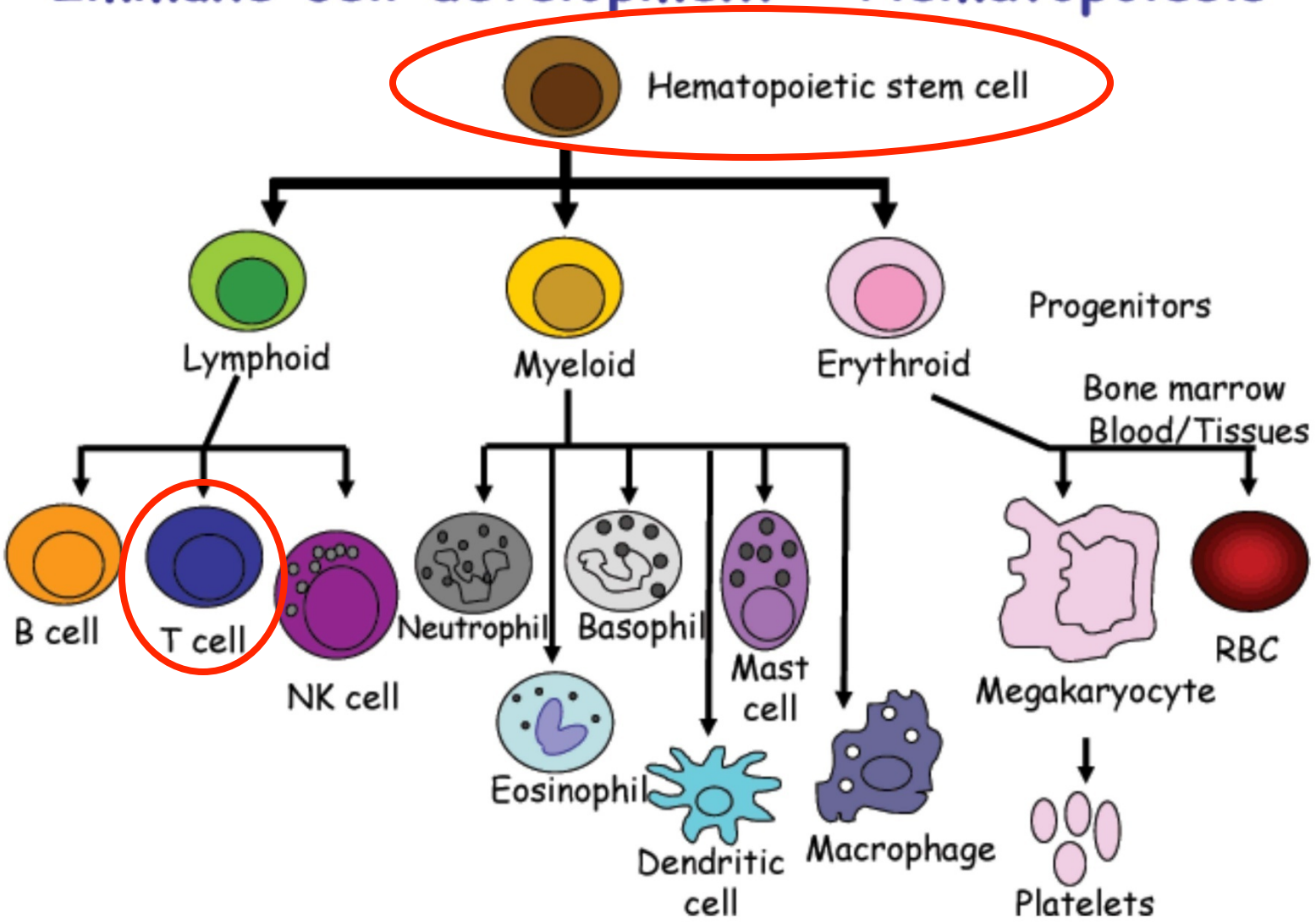
**Gene therapy stages a comeback, albeit a humble one**

Published on May 15, 2009

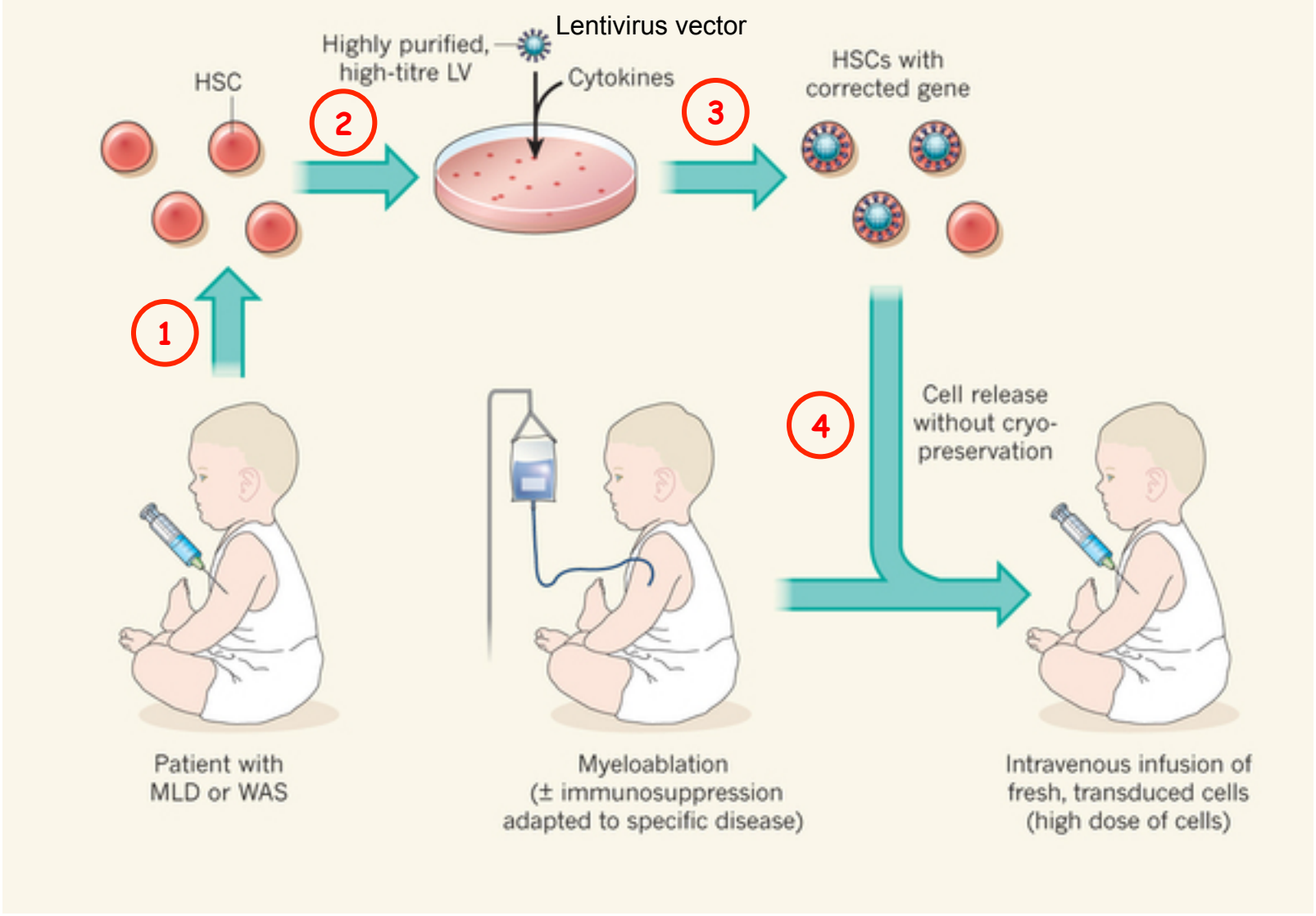
# Improvements in Gene Therapy

- Increases in efficiency of viral transduction
- Higher levels of therapeutic gene expression
- Development of self-inactivating vectors
- Coupling of gene therapy and stem cell technologies

# Immune cell development: Hematopoiesis

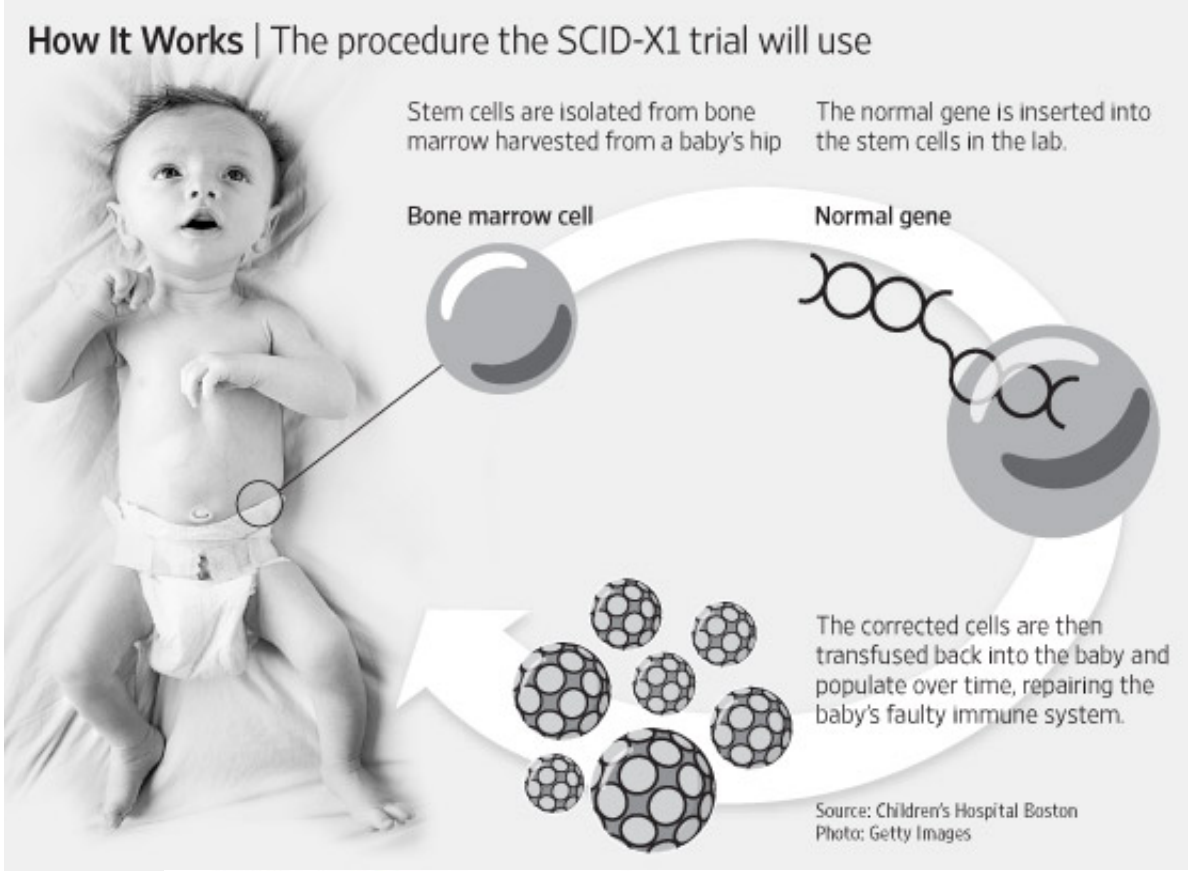


# General Strategy for Use of Hematopoietic Stem Cells in Gene Therapy



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

- **SCID-X1**
  - Most common form of SCID
  - Results from mutations in the common gamma chain gene required for interleukin receptors
  - Patients are immune deficient
- **Gene Therapy Improvements**
  - Used hematopoietic stem cells
  - Improved retroviral vectors with higher titers




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

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

Peter Bracke | November 20, 2014

UCLA

# It Works!

**Gene therapy cures 'bubble boy disease'**

31 Jan 2009, 1128 hrs IST, AP

The new england journal of medicine

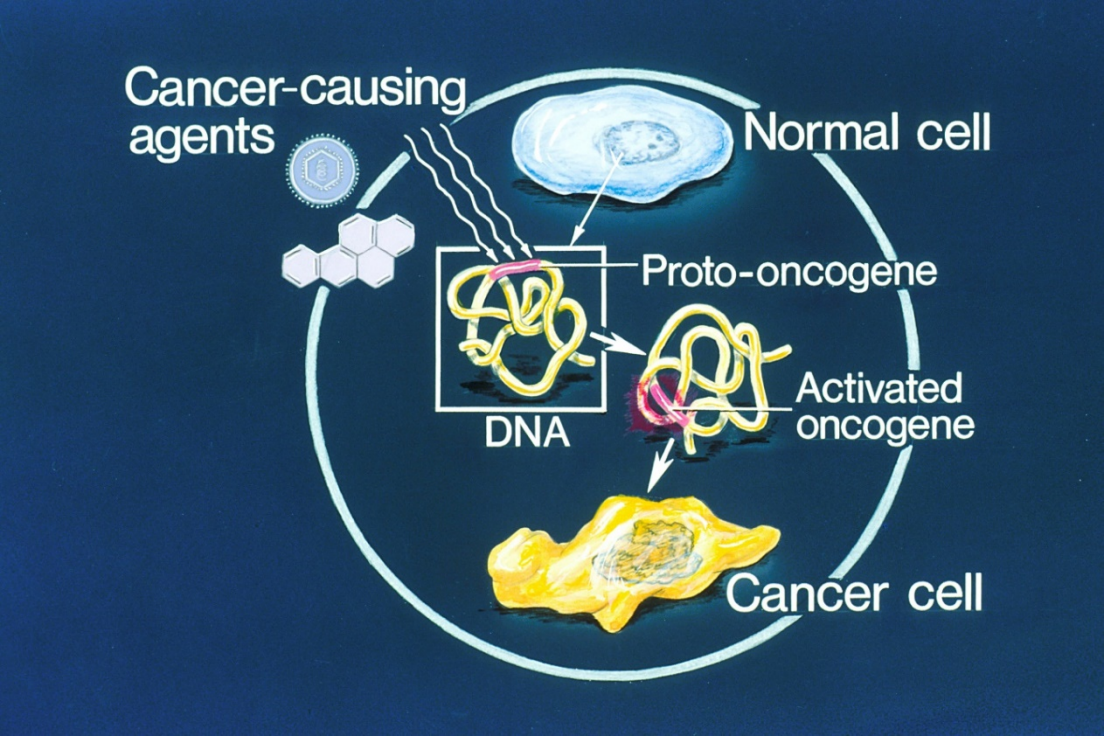
established in 1812 january 29, 2009 vol. 360 no. 5

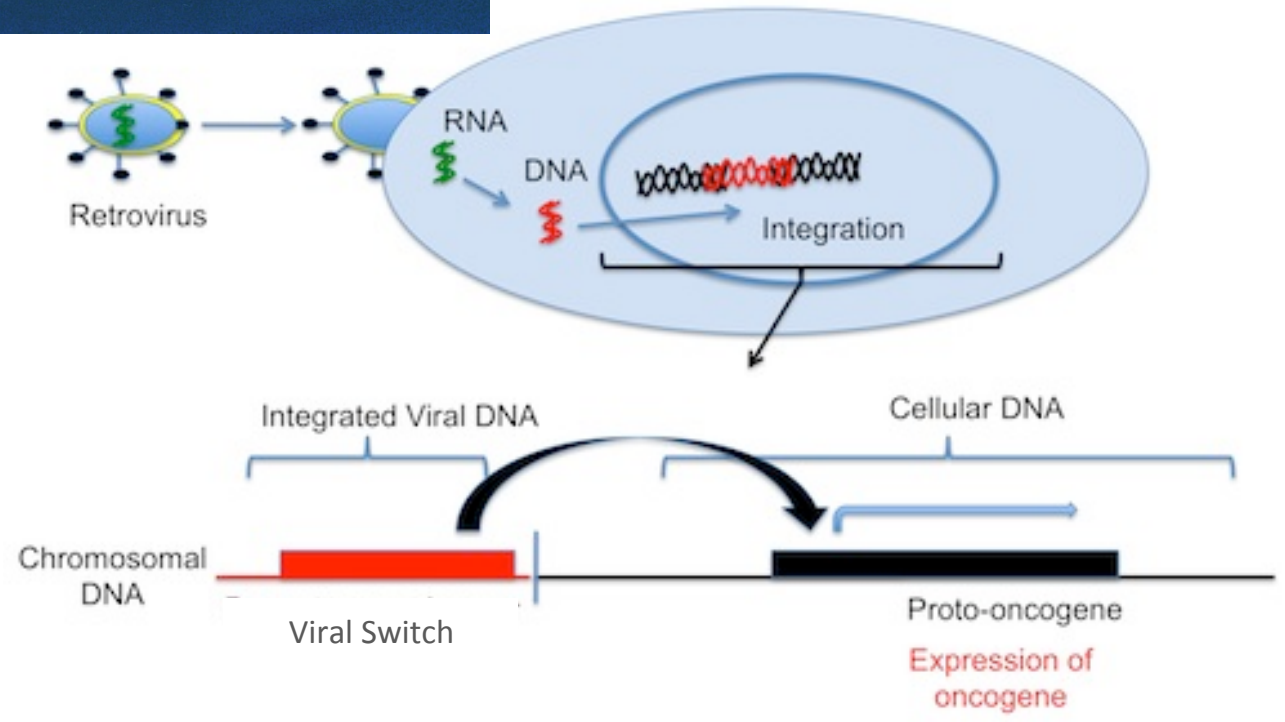
Gene Therapy for Immunodeficiency Due to Adenosine Deaminase Deficiency

## Results after 10 years

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

# Retroviral Switches can Activate Proto-oncogenes and Induce Cancers



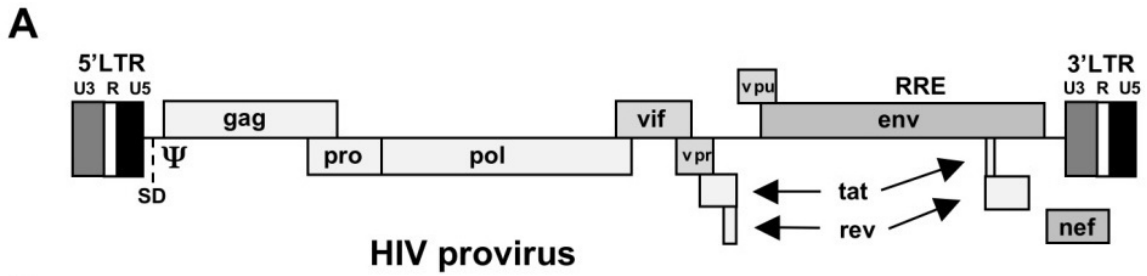

Viral Switch

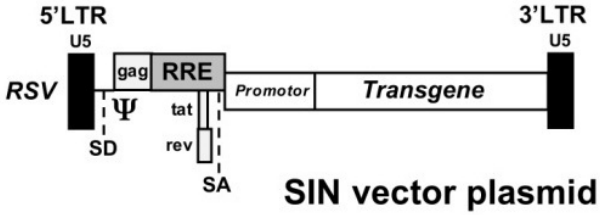
# Development of Self-Inactivating (SIN) Vectors

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

2. The 5' LTR of the viral vector is a powerful switch that can activate proto oncogenes and cause cancers to form.

3. SIN vectors have transcriptionally disabled LTRs. They do not activate adjacent genes.



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

ScienceDaily®
Your source for the latest research news

Mobile: iPhone Android Web Follow: Facebook Twitter Google+

HEALTH | PHYSICAL/TECH | ENVIRONMENT | SOCIETY/EDUCATION | QUIRKY

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

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

**Date:** December 8, 2013

**Source:** Dana-Farber/Boston Children's Cancer and Blood Disorders Center

**Summary:** Researchers reported promising outcomes data for the first group of boys with X-linked severe combined immunodeficiency syndrome, a fatal genetic immunodeficiency also known as "bubble boy" disease, who were treated as part of an international clinical study of a new form of gene therapy. Its delivery mechanism was designed to prevent the leukemia that arose a decade ago in a similar trial in Europe.

**Share This**

- Email to a friend
- Facebook
- Twitter
- Google+
- Print this page
- More options

"Eight of the nine boys registered to date in the new trial are alive and well, with functioning immune systems and free of infections associated with SCID-X1, between nine and 36 months following treatment".

**Fischer et al. 2015**

Table 1. PID diseases and gene therapy

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

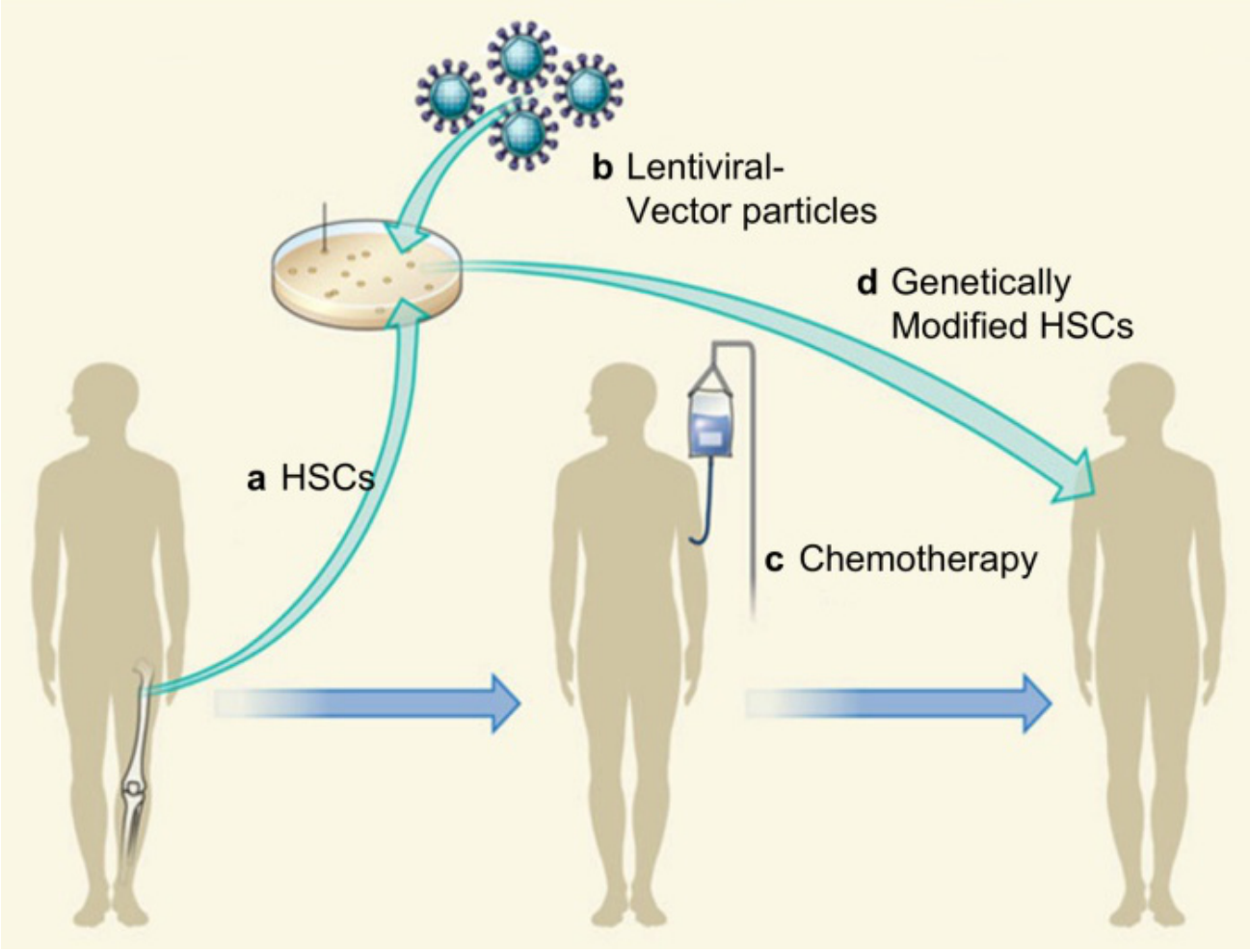
ADA, adenosine deaminase; HLH, hemophagocytic lymphohistiocytosis; IPEX, immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome; PID, primary immunodeficiencies; SAEs, serious adverse events; SCID, severe combined immunodeficiencies; SIN, self-inactivating; WAS, Wiskott–Aldrich syndrome.

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

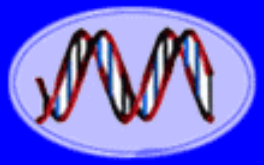
[b]Associated with very high frequency of SAEs (seven out of nine for WAS, and four out of four for CGD).

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

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

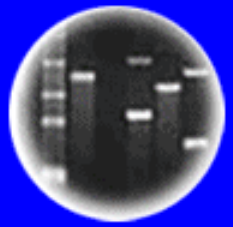



- ADA-SCID
- Chronic granulomatous disease
- Leucocyte adhesion deficiency
- SCID Artemis
- SCID Rag-1
- SCID-X1
- Sickle cell disease
- β-thalassaemia
- Wiskott Aldrich Syndrome
- X-linked lymphoproliferative syndrome



DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



DNA Fingerprinting

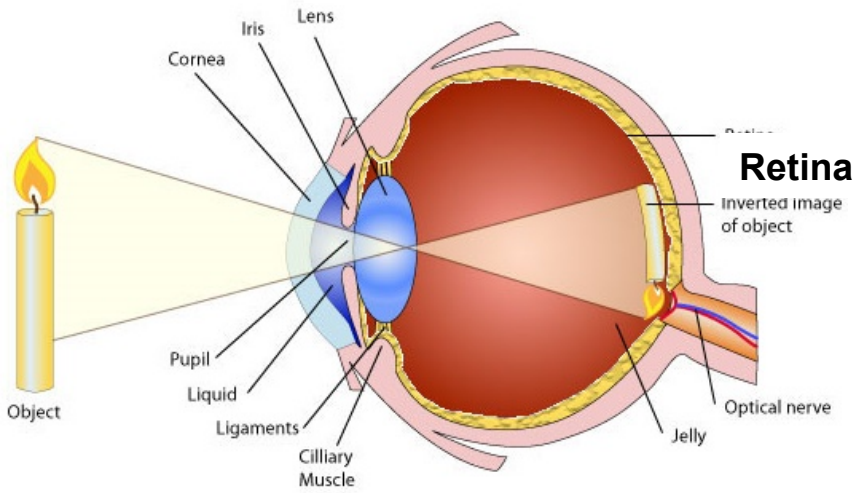
Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

# In Vivo
# Gene Therapy

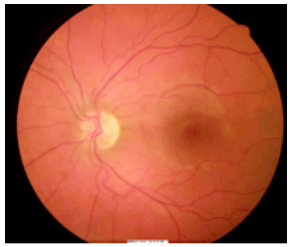
# Blindness – Choroideremia (CHM)

## How We See

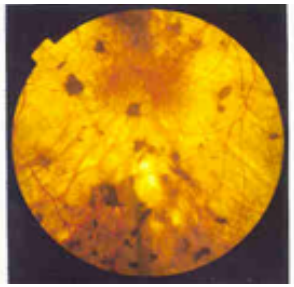


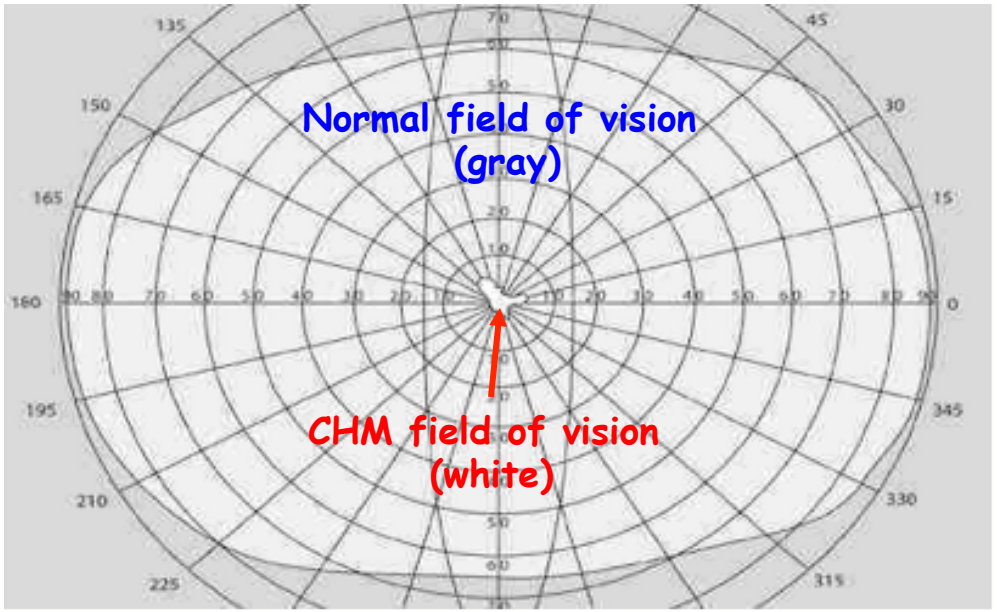

1. CHM is a rare inherited cause of blindness that affects around 1 in 50,000 people. Night blindness is an early symptom.
2. CHM is caused by mutation in the X-linked REP1 gene.
3. Without the REP1 protein, pigment cells in the retina die prematurely

**Normal retina**



**CHM Retina**





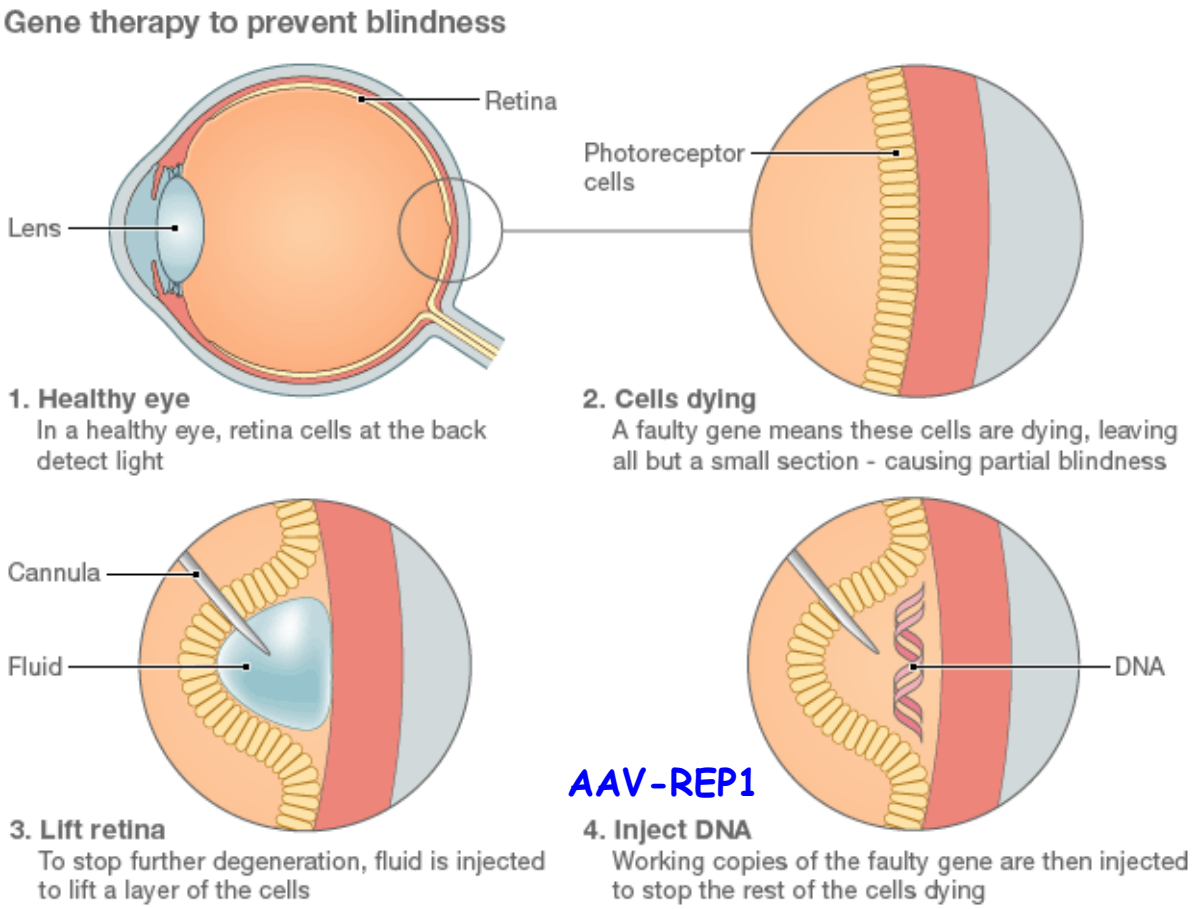
BBC NEWS

HEALTH

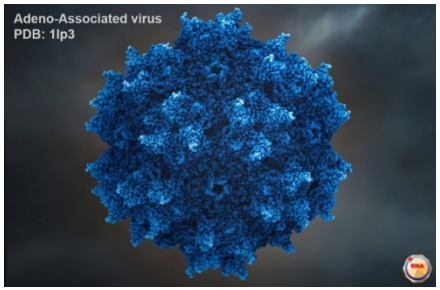
UNIVERSITY OF OXFORD

**15 January 2014** Last updated at 20:55 ET

# Gene therapy 'could be used to treat blindness'




**AAV-REP1**




## Adeno-associated viruses (AAV)

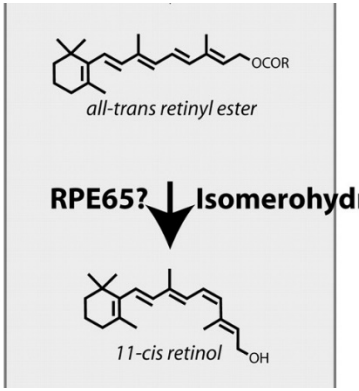
- Does not generally provoke antibody formation
- Infects nondividing cells of many different tissues
- Little or no integration of viral DNA into the host genome

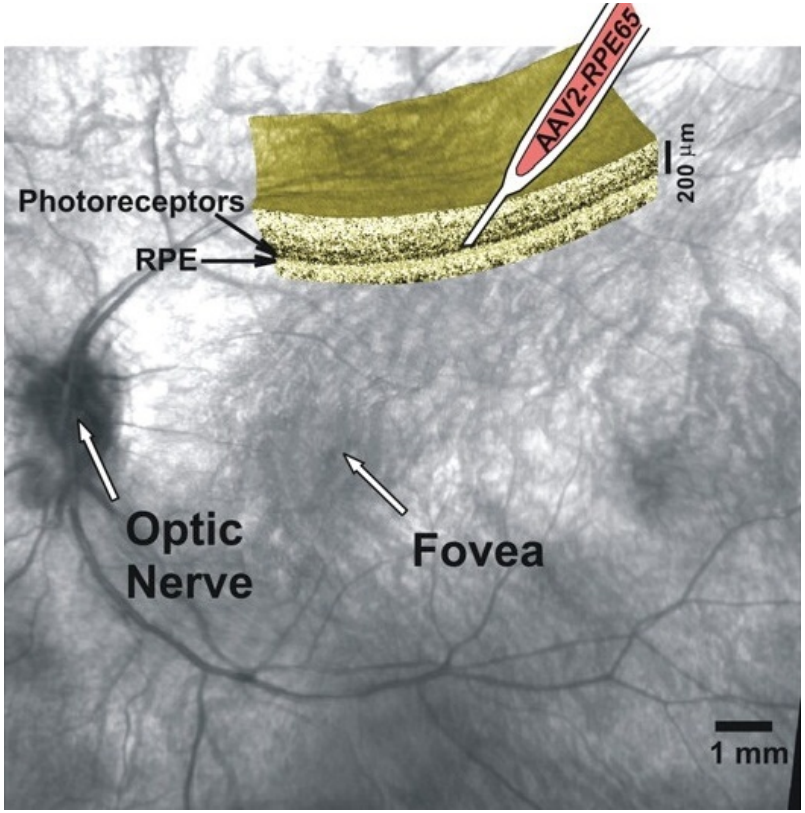
Jonathan Wyatt, one of six patients whose vision improved as a result of REP1 gene therapy

# LCA Gene Therapy Using RPE65 & AAV2

Leber Congenital Amaurosis
- Degenerative diseases of the retina
- The most common cause of congenital blindness in children

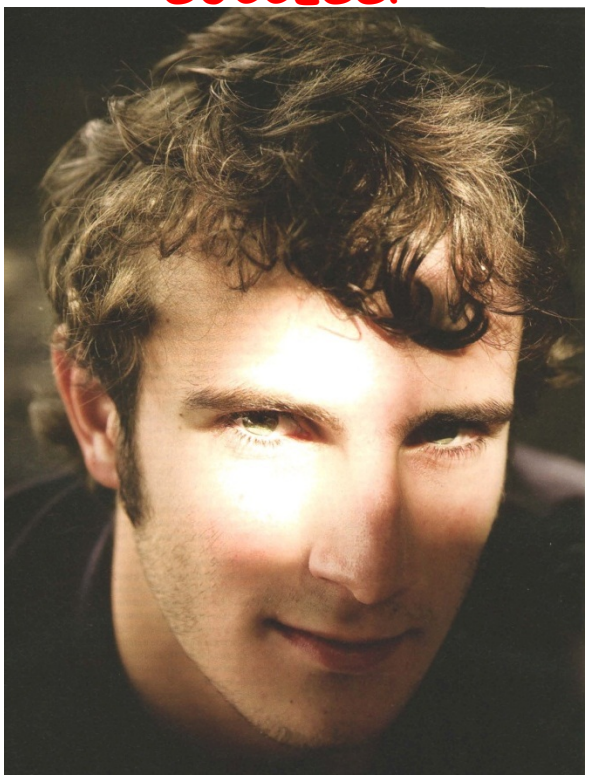
Type 2 LCA is caused by recessive mutations in the RPE65 isomerase gene that recycles photoreceptors




Cideciyan et al. PNAS 2008;105:15112

SUCCESS!



ALESSANDRO CANNATA

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

SCIENCE

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

By ANDREW POLLACK OCT. 5, 2015

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

The NEW ENGLAND JOURNAL of MEDICINE
May 14, 2015

BRIEF REPORT

# Improvement and Decline in Vision with Gene Therapy in Childhood Blindness

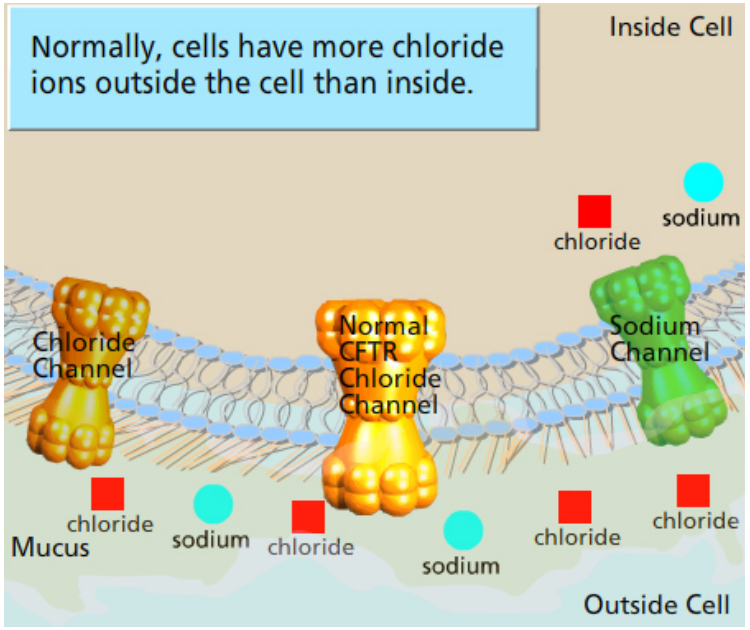
Samuel G. Jacobson, M.D., Ph.D., Artur V. Cideciyan, Ph.D., Alejandro J. Roman, M.Sc., Alexander Sumaroka, Ph.D., Sharon B. Schwartz, M.S., C.G.C., Elise Heon, M.D., and William W. Hauswirth, Ph.D.

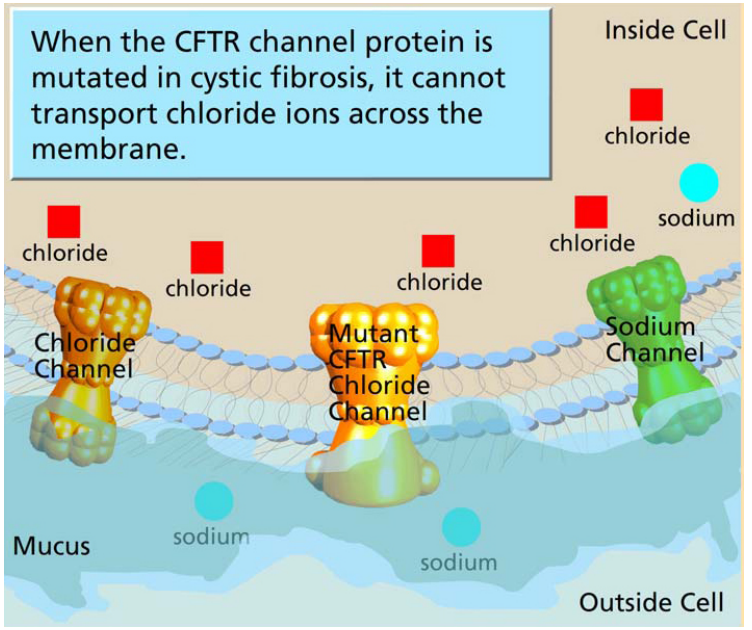
SUMMARY

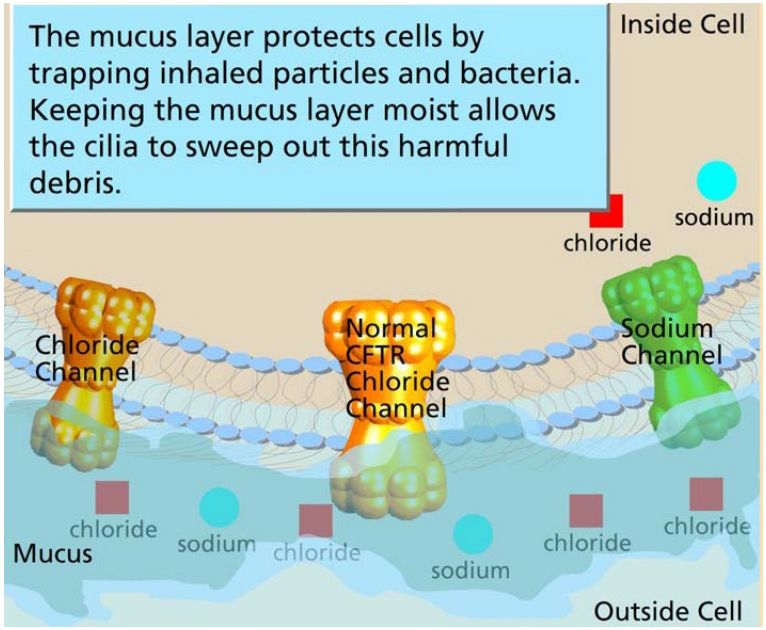
Retinal gene therapy for Leber's congenital amaurosis, an autosomal recessive childhood blindness, has been widely considered to be safe and efficacious. Three years after therapy, improvement in vision was maintained, but the rate of loss of photoreceptors in the treated retina was the same as that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

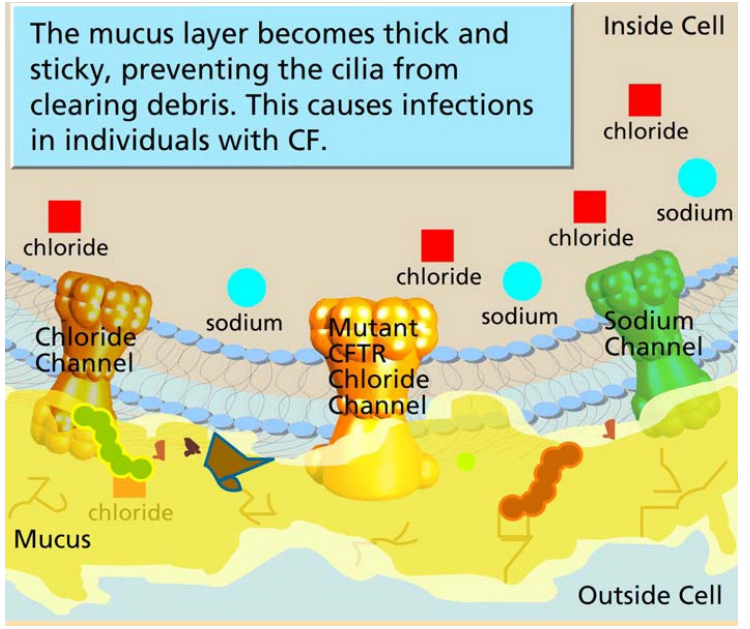
# Cystic Fibrosis Results from a Defect in a Chloride Channel

Normal



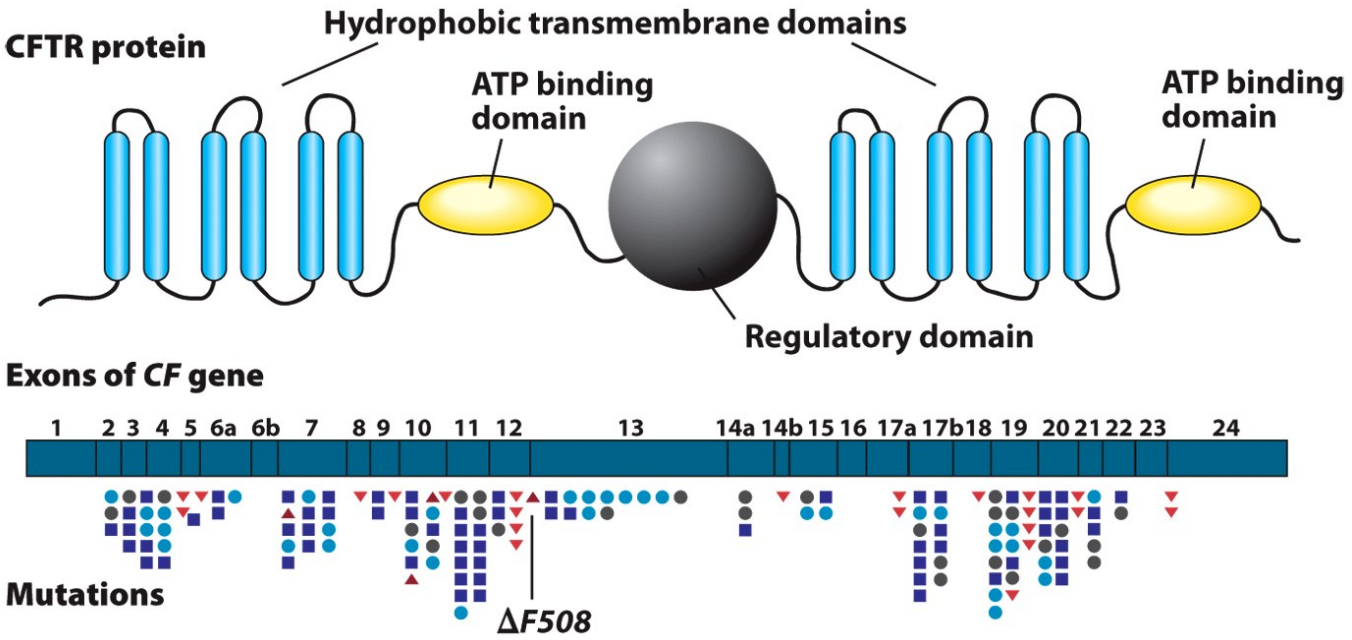




Diseased

http://learn.genetics.utah.edu/content/tech/genetherapy/cysticfibrosis/index.html

# Mutant Cystic Fibrosis Genes
## [Recessive (Loss-Of-Function) Mutations]



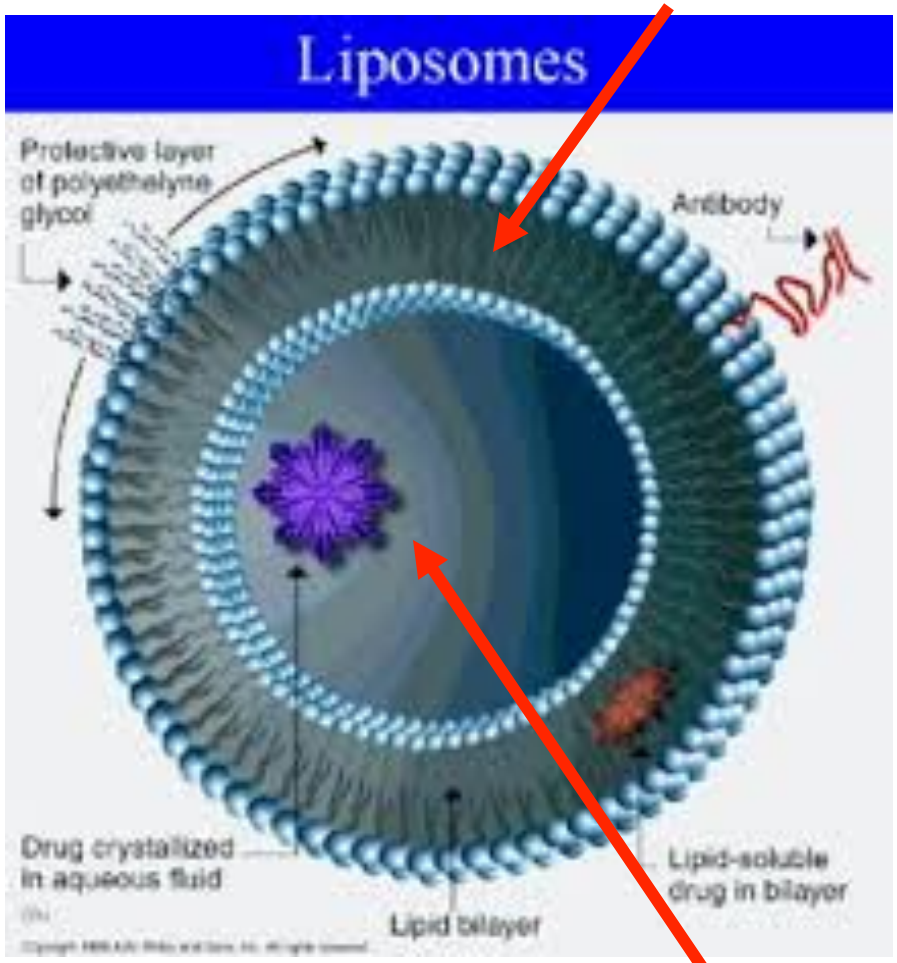

- Mostly N. European Ancestry
- 1/2500 CF Babies Born in US/Year
- 1/20 Americans are CF Carriers
- 30 Year Life Span
- Abnormality of Mucus/Sweat Glands
- Cannot Regulate Salt (Chlorides)

70% of Families Have ΔF508 Deletion-What Are Consequences For CF Testing? How Can it be in 70% of CF Families?

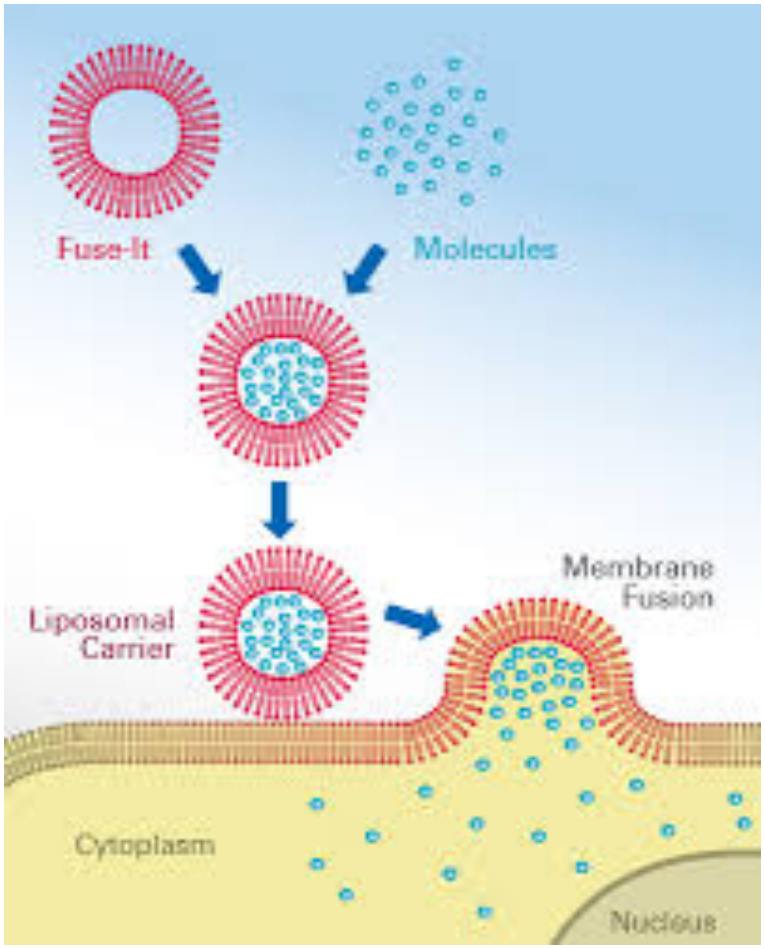
Key:
- In-frame deletion
- Missense mutation
- Nonsense mutation
- Frame-shift mutation
- Splicing mutation

# Liposome-based Delivery of the Cystic Fibrosis Gene to Lung Cells

Lipid bilayer similar to cell membrane

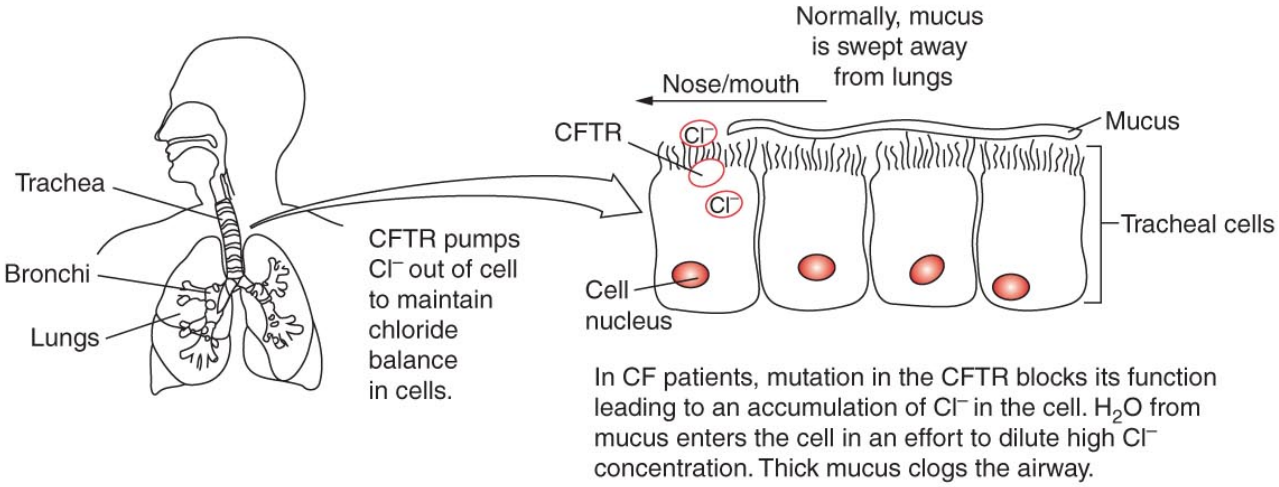


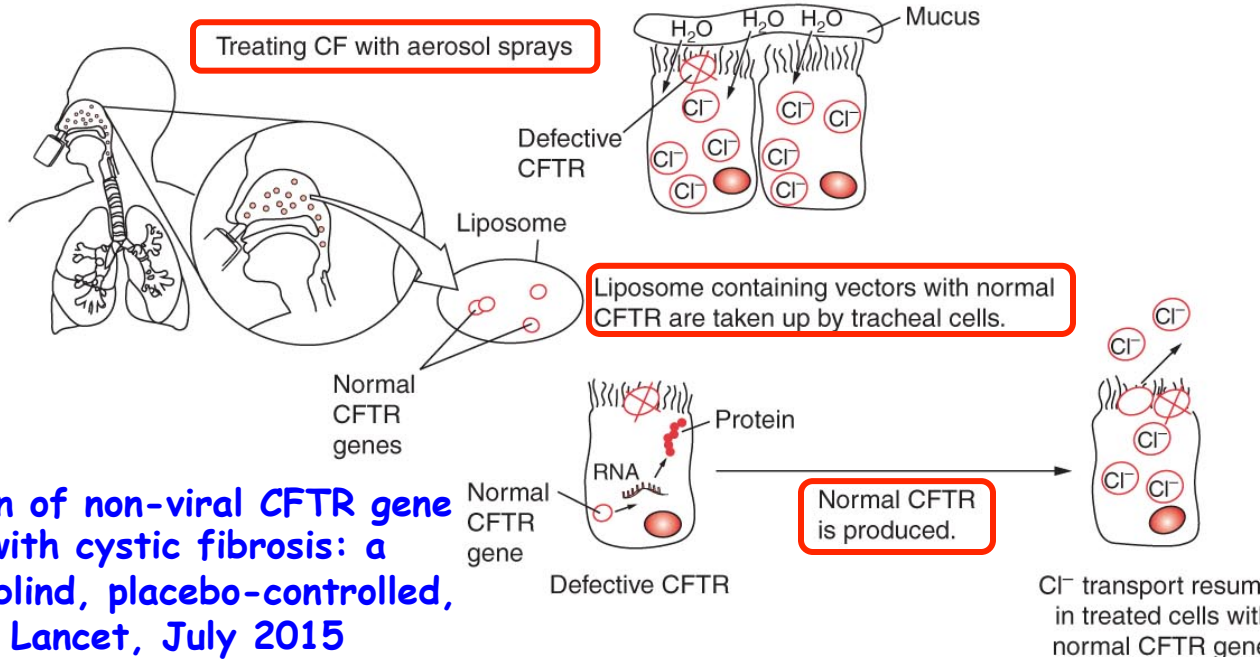

Delivery of CFTR genes to lung cells




CFTR genes are sequestered in liposome

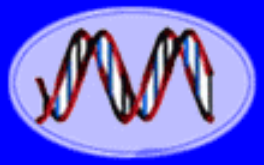
# In Vivo Cystic Fibrosis Gene Therapy



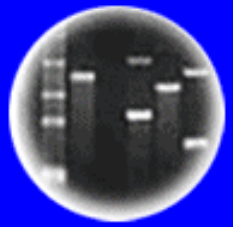

**Repeated nebulisation of non-viral CFTR gene therapy in patients with cystic fibrosis: a randomised, double-blind, placebo-controlled, phase 2b trial – The Lancet, July 2015**

**We noted a significant, albeit modest, treatment effect in the pGM169/GL67A group versus placebo at 12 months' follow-up. This outcome was associated with a stabilisation of lung function in the pGM169/GL67A group compared with a decline in the placebo group.**



DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



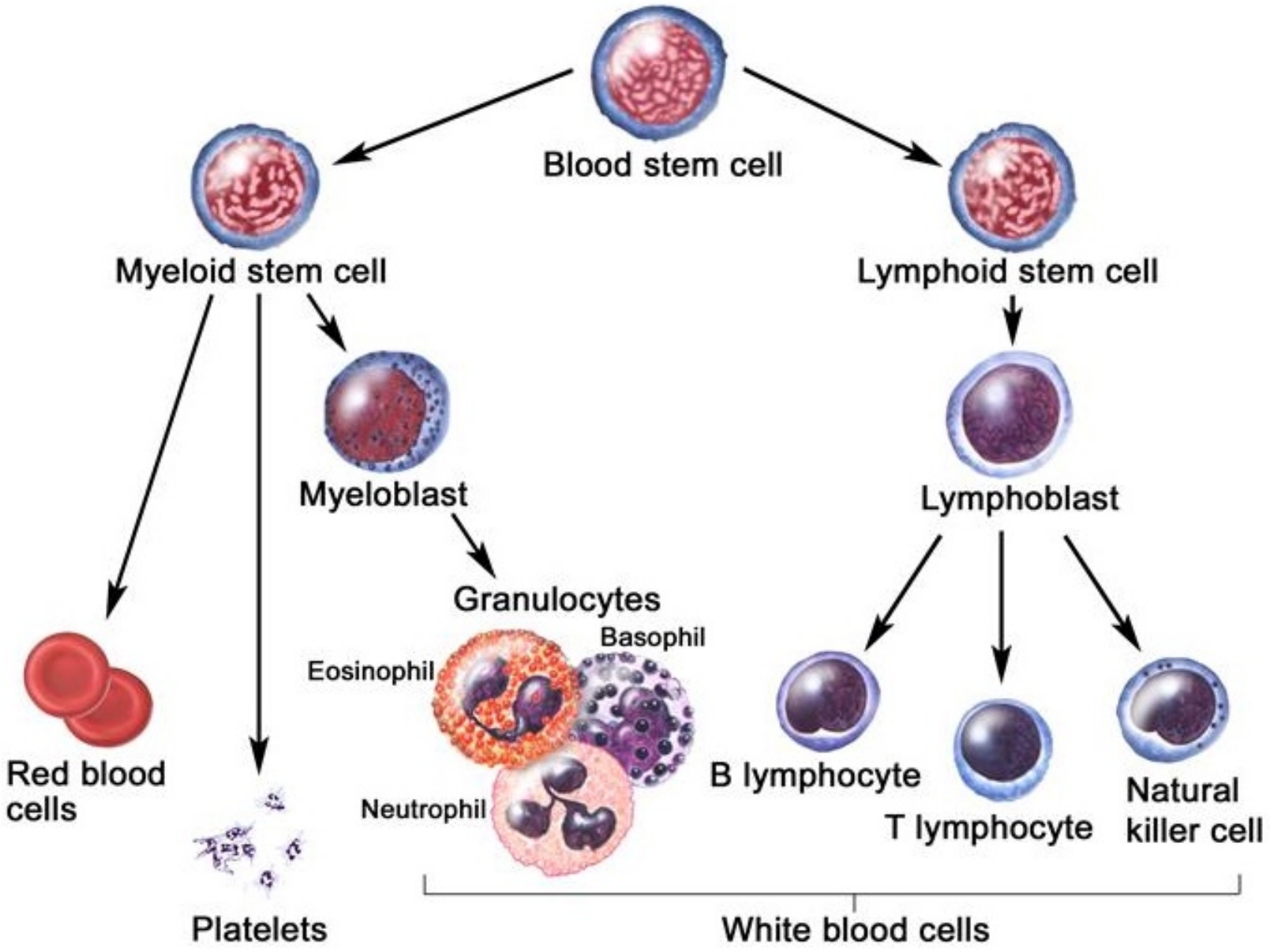
DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

# Gene Therapy to Control Cancers

# Normal Blood Cell Development



The New York Times

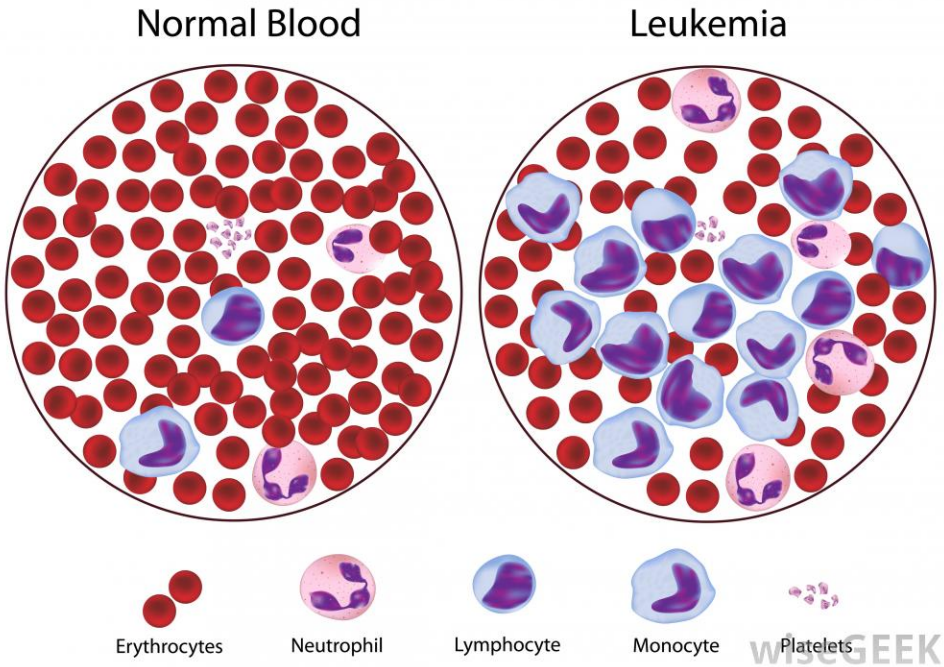
December 9, 2012

2013

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

By DENISE GRADY


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

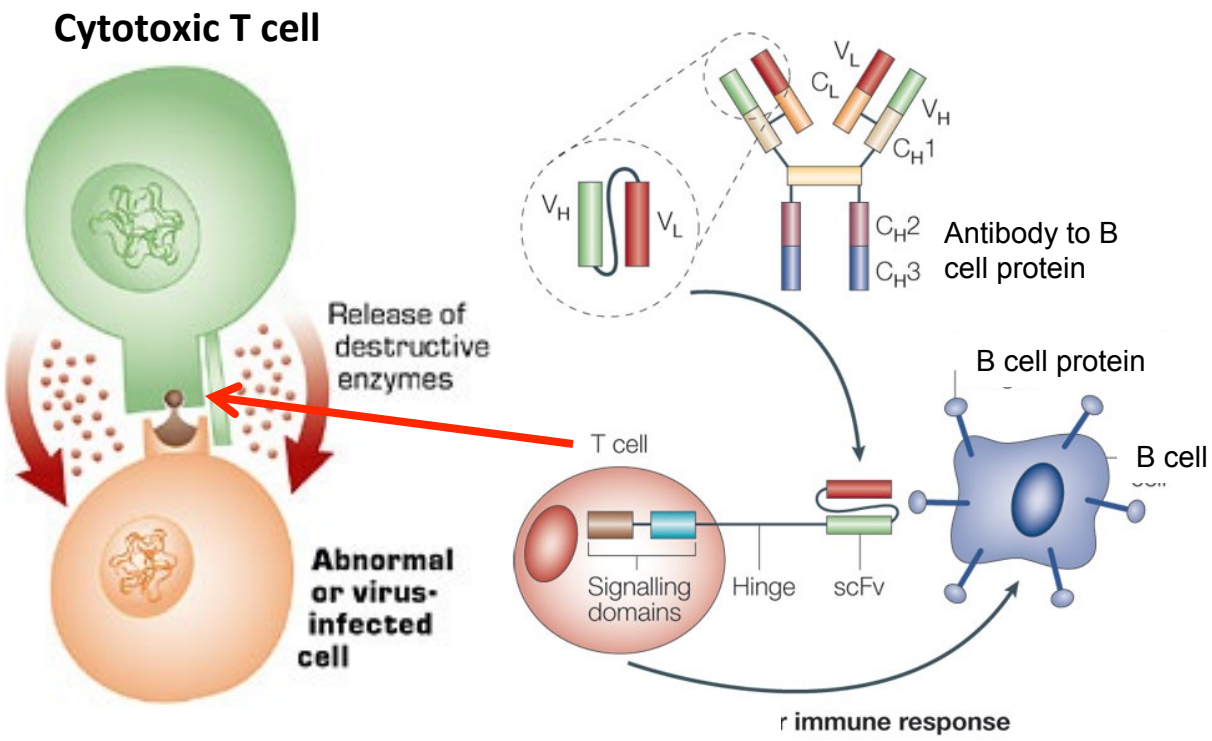



*Emily Whitehead, 7, was the first child to receive gene therapy for leukemia at CHOP. (Photo courtesy of The Children's Hospital of Philadelphia)*

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

# Ex-vivo Gene Therapy for Lymphocytic Leukemia

Science Translational Medicine
AAAS




## Protocol

- Removed T cells from patients
- Created gene encoding Chimeric Antigen Receptor (CAR) that recognize a protein on the surface of B cells
- Transferred CAR genes into T cells to allow them to target B cells
- Infused CAR T cells back into patients

## Results

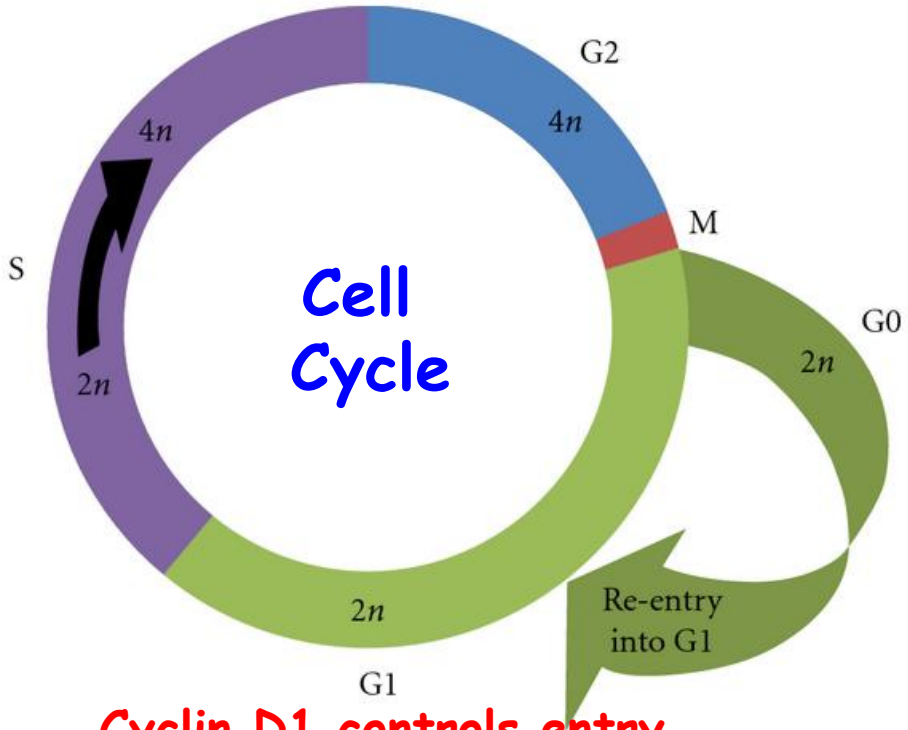
- CAR T cells expanded more than 1,000 fold and persisted more than six months
- Estimated that each CAR T cell killed more than 1,000 cancer cells
- In one trial, 19 of 22 children who had exhausted all drug treatment and bone-marrow transplant options for leukemia went into remission after receiving CART-19
- 45 of 75 leukemia patients saw complete regressions with CARs

# Silencing Genes to Control Cancer

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

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

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



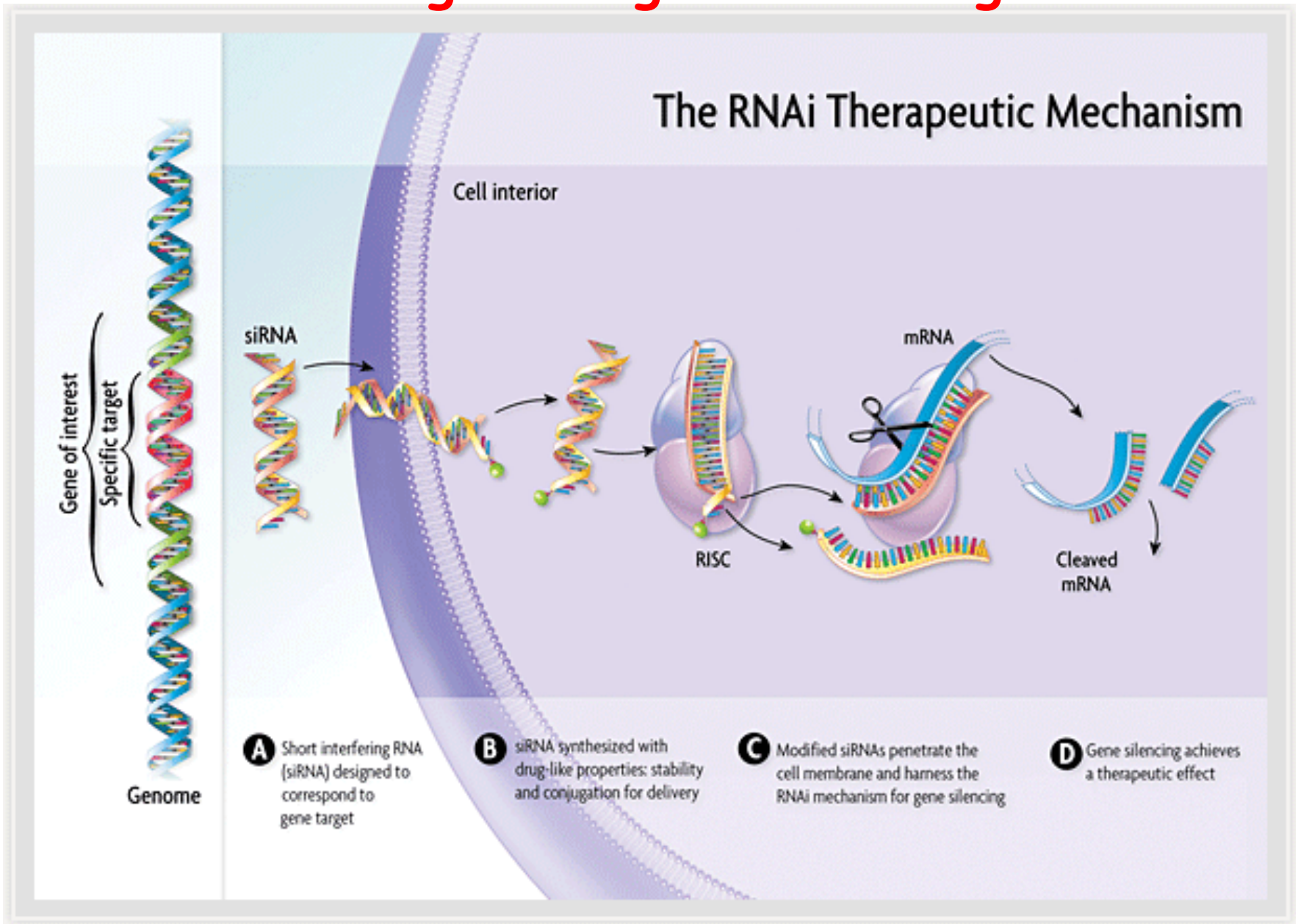

Cyclin D1 controls entry into DNA synthesis phase

### Gene Therapy Approach

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

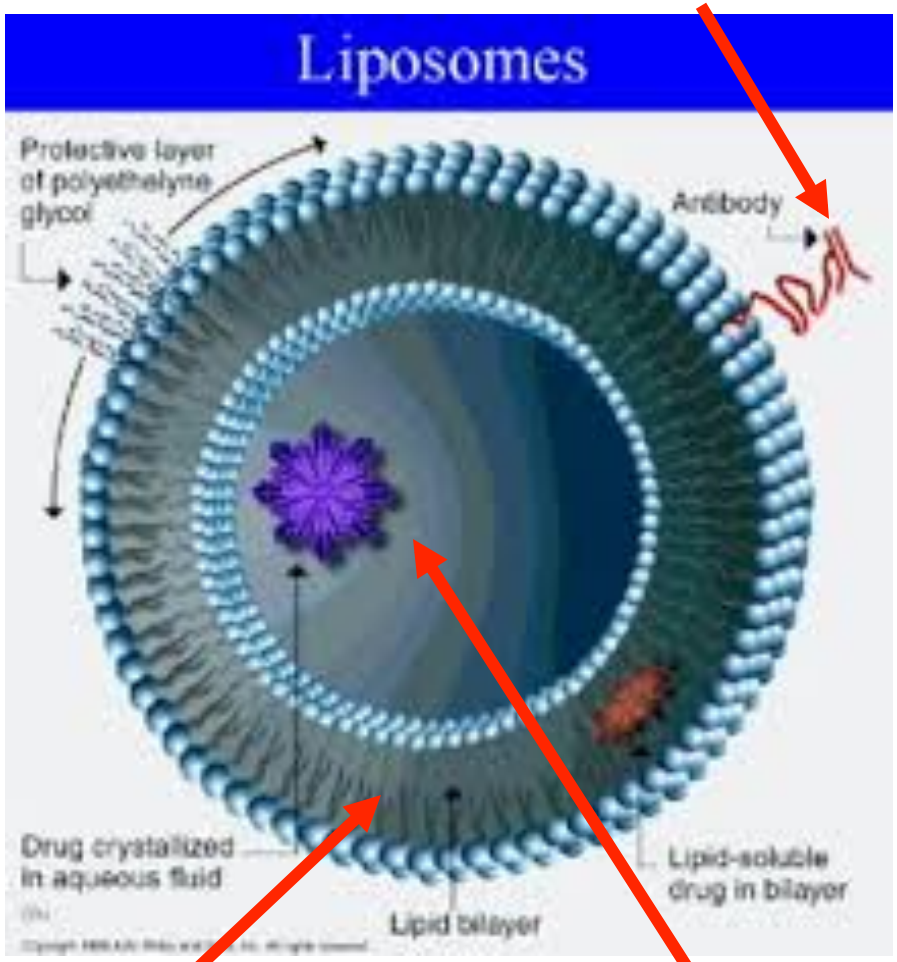
# RNA Interference

## Gene Silencing Through mRNA Degradation

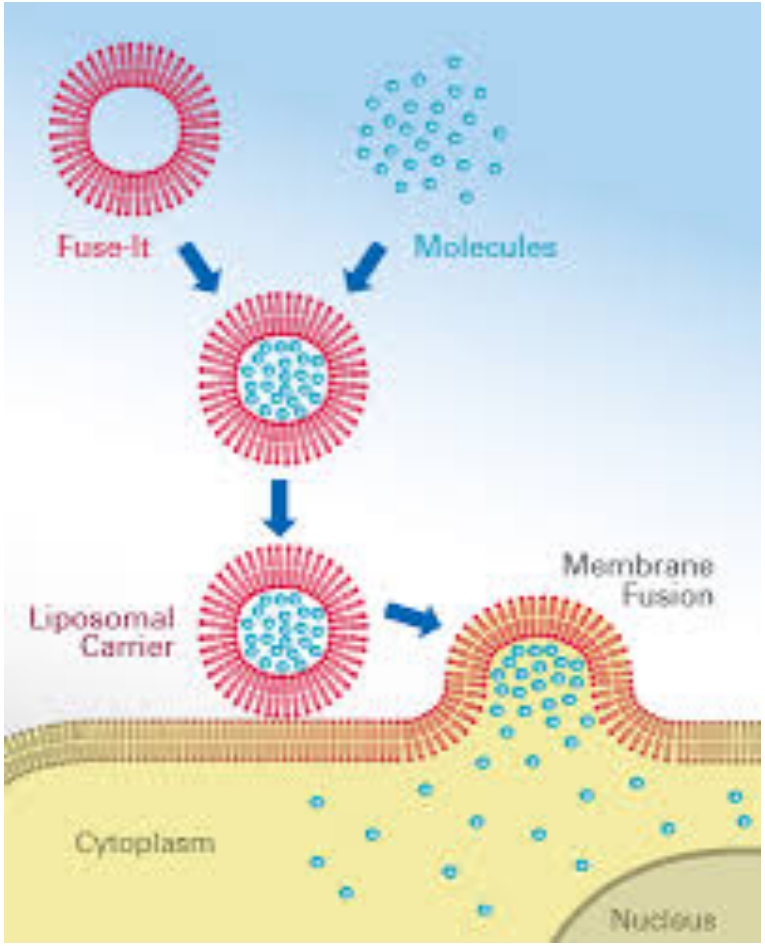


# Lipid-based Nanoparticle for Specific Delivery of siRNA to MCL Cells *in Vivo*

Antibody that binds MCL cells

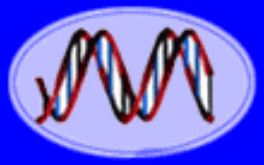



Delivery of siRNAs to MCL cell



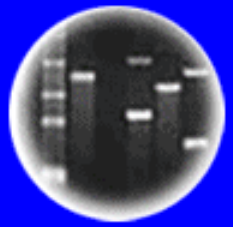

Lipid bilayer similar to cell membrane

siRNAs are sequested in liposome



DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

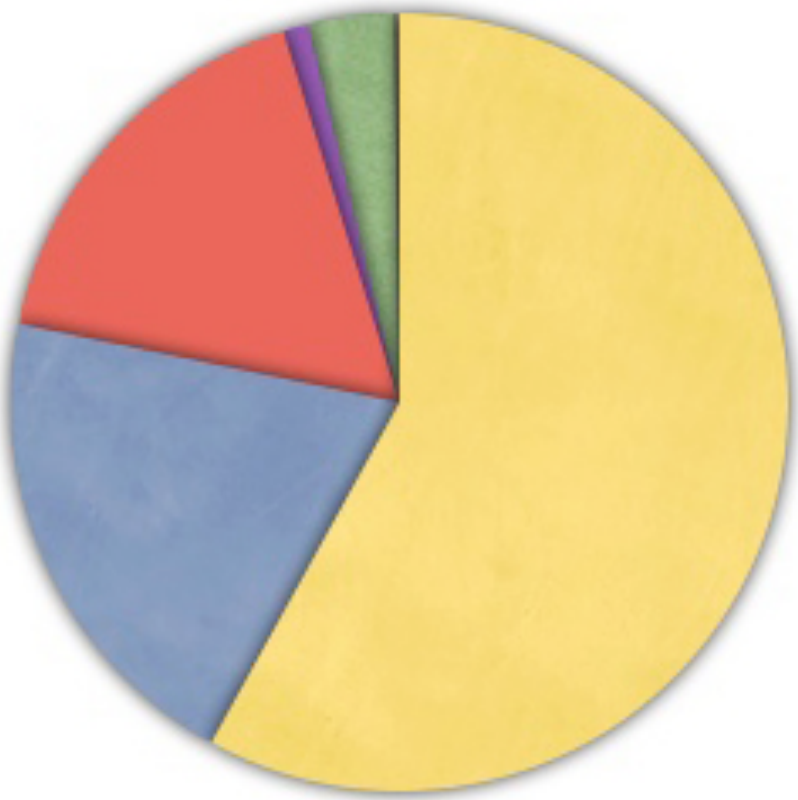
Plants of Tomorrow

# Current Status of Gene Therapy

# Clinical Trials

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

# Phases of Gene Therapy Clinical Trials

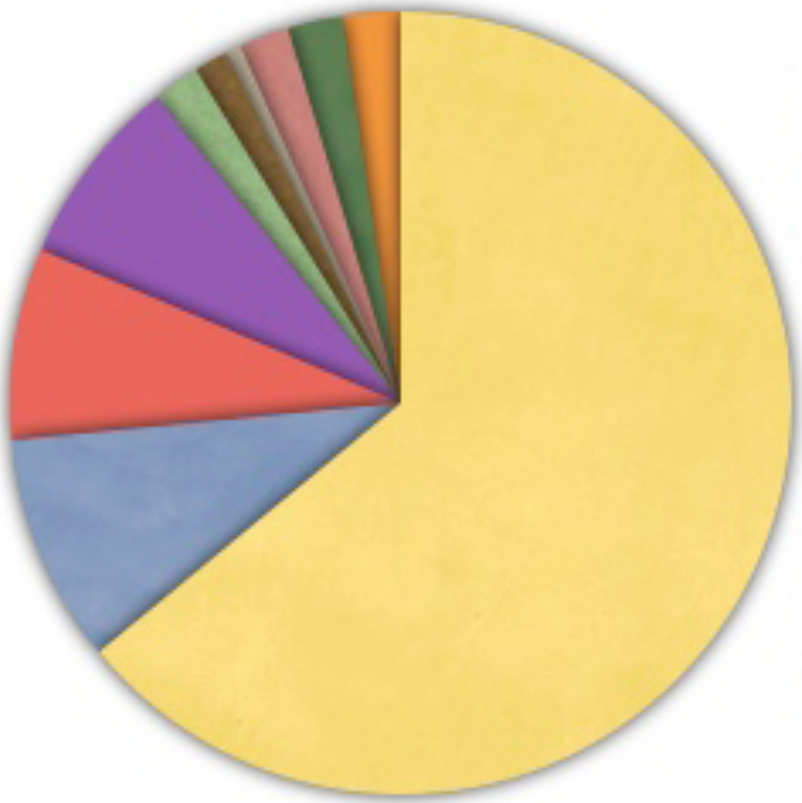


- Phase I 58.1% (n=1283)
- Phase I/II 20.2% (n=446)
- Phase II 16.9% (n=374)
- Phase II/III 1% (n=23)
- Phase III 3.6% (n=79)
- Phase IV 0.1% (n=2)
- Single subject 0.1% (n=3)



www.wiley.co.uk/genmed/clinical

# Indications Addressed by Gene Therapy Clinical Trials



- Cancer diseases 64% (n=1415)
- Monogenic diseases 9.5% (n=209)
- Cardiovascular diseases 7.9% (n=175)
- Infectious diseases 7.9% (n=174)
- Neurological diseases 1.9% (n=43)
- Ocular diseases 1.4% (n=31)
- Inflammatory diseases 0.6% (n=14)
- Other diseases 2.1% (n=46)
- Gene marking 2.3% (n=50)
- Healthy volunteers 2.4% (n=53)



www.wiley.co.uk/genmed/clinical

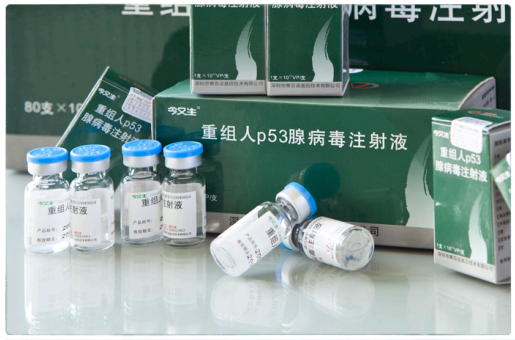
# Approved Gene Therapy Products Worldwide

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

赛百诺 SIBIONO

**Gendicine is a genetically engineered, infectious active recombinant human p53 adenovirus particles (rAd-p53), the replication-defective adenovirus type 5 and human p53 tumor suppressor gene normally composed of two parts, a replication-defective adenovirus particles as a carrier of the p53 gene into tumor cells, p53 gene expression in tumor cells of p53 protein plays inhibit tumor cell growth and induced apoptosis of tumor cells, inhibiting the biological function of tumor angiogenesis and bystander effects.**

**Marketed 2004**




uniQure

Glybera® (alipogene tiparvovec) overview

Glybera is a gene therapy that is designed to restore the LPL enzyme activity required to enable the processing, or clearance, of fat-carrying chylomicron particles formed in the intestine after a fat-containing meal. The product consists of an engineered copy of the human LPL gene packaged with a tissue-specific promoter in a non-replicating AAV1 vector, which has a particular affinity for muscle cells. In order to improve activity, uniQure uses a naturally occurring variant of the LPL gene that has higher enzyme activity than the normal version of the gene that encodes the protein. The company produces Glybera using its insect cell-based manufacturing process. Clinicians administer Glybera in a one-time series of up to 60 intramuscular injections in the legs. The patient is administered spinal anesthesia or deep sedation during the procedure. In addition, an immunosuppressive regimen is recommended from three days prior to and for 12 weeks following Glybera administration.

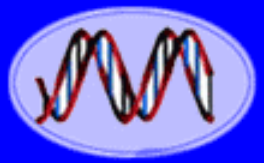
**Marketed 2012**

FDAnews Drug Daily Bulletin

**Pharmaceuticals / Submissions and Approvals**

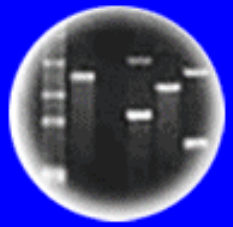
## UniQure Won't Seek U.S. Regulatory Approval for Glybera Gene Therapy Program

Dec. 9, 2015



DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

# Regulation & Issues Concerning Gene Therapy

# US Regulatory Authority for Gene Therapy

- Department of Health and Human Services (DHHS) has been charged with oversight of clinical trials
  - Office for Human Research Protections
    - All research involving human subjects undergo Institutional Review Board review
  - U.S. Food and Drug Administration
    - Center for Biologics Evaluation and Research regulates human gene therapies. Manufacturers of gene therapy products must test their products extensively and meet FDA requirements for safety, purity and potency before they can be sold in the United States

- National Institutes of Health (NIH), oversees the conduct of federally funded clinical trials
  - Recombinant DNA Advisory Committee review human gene transfer research on behalf of the NIH through the Office of Biotechnology Activities

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

# Gene Therapy for Human Genetic Disease?

3 March 1972, Volume 175, Number 4025

SCIENCE

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

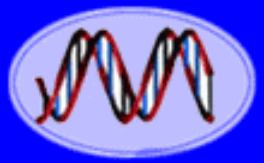
Theodore Friedmann and Richard Roblin

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

1. There should be adequate biochemical characterization of the prospective patient's genetic disorder.
2. There should be prior experience with untreated cases of what appears to be the same genetic defect
3. There must be an adequate characterization of the quality of the exogenous DNA vector.
4. There should be extensive studies in experimental animals to evaluate the therapeutic benefits and adverse side effects of the prospective techniques.
5. Where possible, determine whether the prospective gene therapy technique can restore enzyme function in the cells of the prospective patient

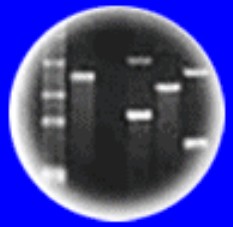
# Some Issues With Human Gene Therapy

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



DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



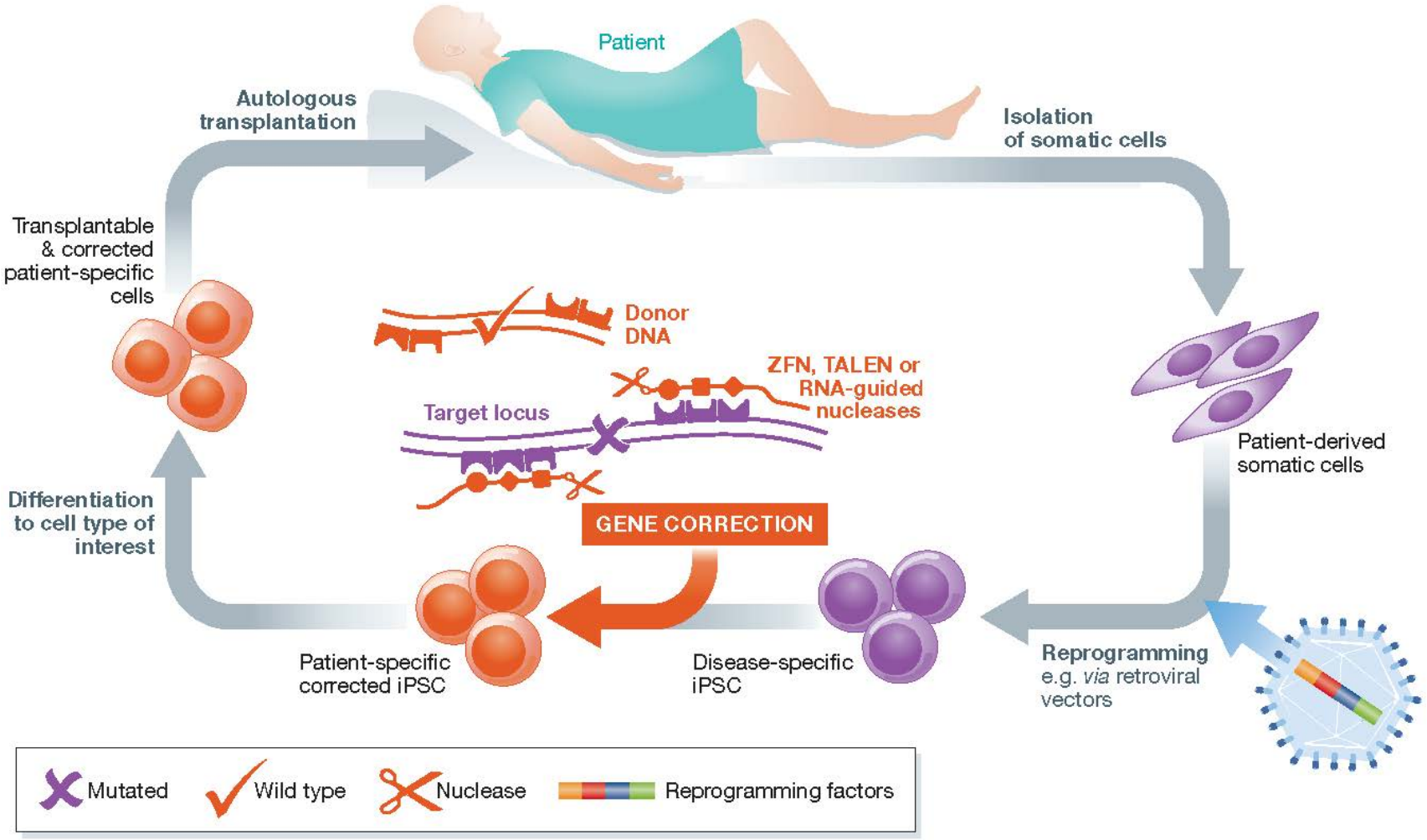
DNA Fingerprinting

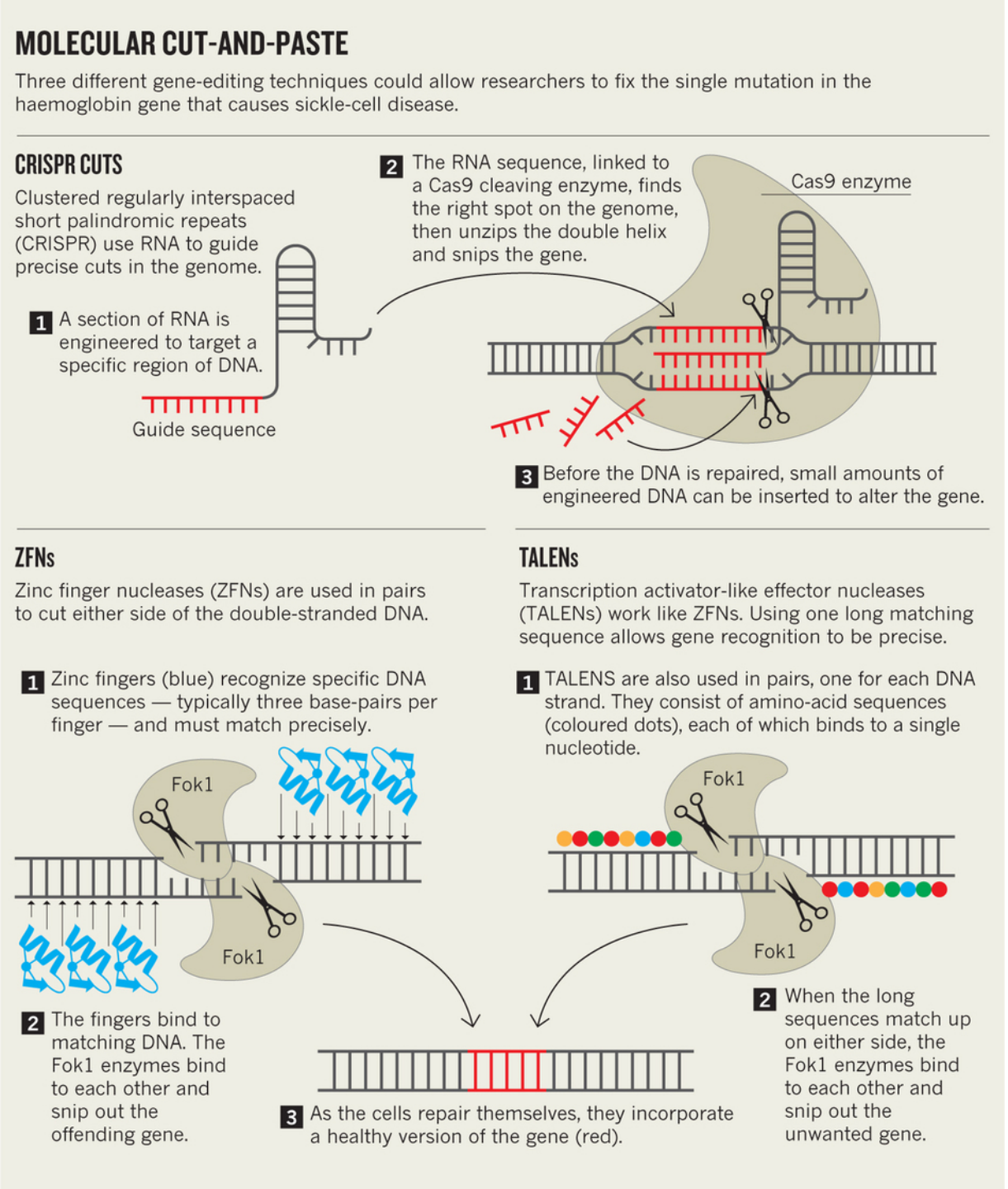
Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

# Gene Editing & Human Gene Therapy

# Human Genome Editing Therapy






# Approaches to Specifically Edit the Genome

- DNA editing nucleases introduce double-stranded breaks in DNA
- DNA sequences can be specifically altered during the repair of these breaks
- Genes can be specifically targeted to become inactivated, altered, or added to the genome.

# Uses of Genome Editing

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

Xiao-Jie et al. 2015

# The Future is Now for Human Genome Editing Therapy

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

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

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

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

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

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

TRANSGENIC ANIMALS

**Editing of Targeted Genes Proved Possible in Monkeys**

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

05 November 2015

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

Brief Report  Switch to Standard

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

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

[7] These authors contributed equally to this work

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

Brief Report  Switch to Standard

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

Gerald Schwank[7], Bon-Kyoung Koo[78], Valentina Sasselli, Johanna F. Dekkers, Inha Heo, Turan Demircan, Nobuo Sasaki, Sander Boymans, Edwin Cuppen, Cornelis K. van der Ent, Edward E.S. Nieuwenhuis, Jeffrey M. Beekman, Hans Clevers

[7] These authors contributed equally to this work

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

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

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

+ Author Affiliations

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

* These authors contributed equally to this work.

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

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

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

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

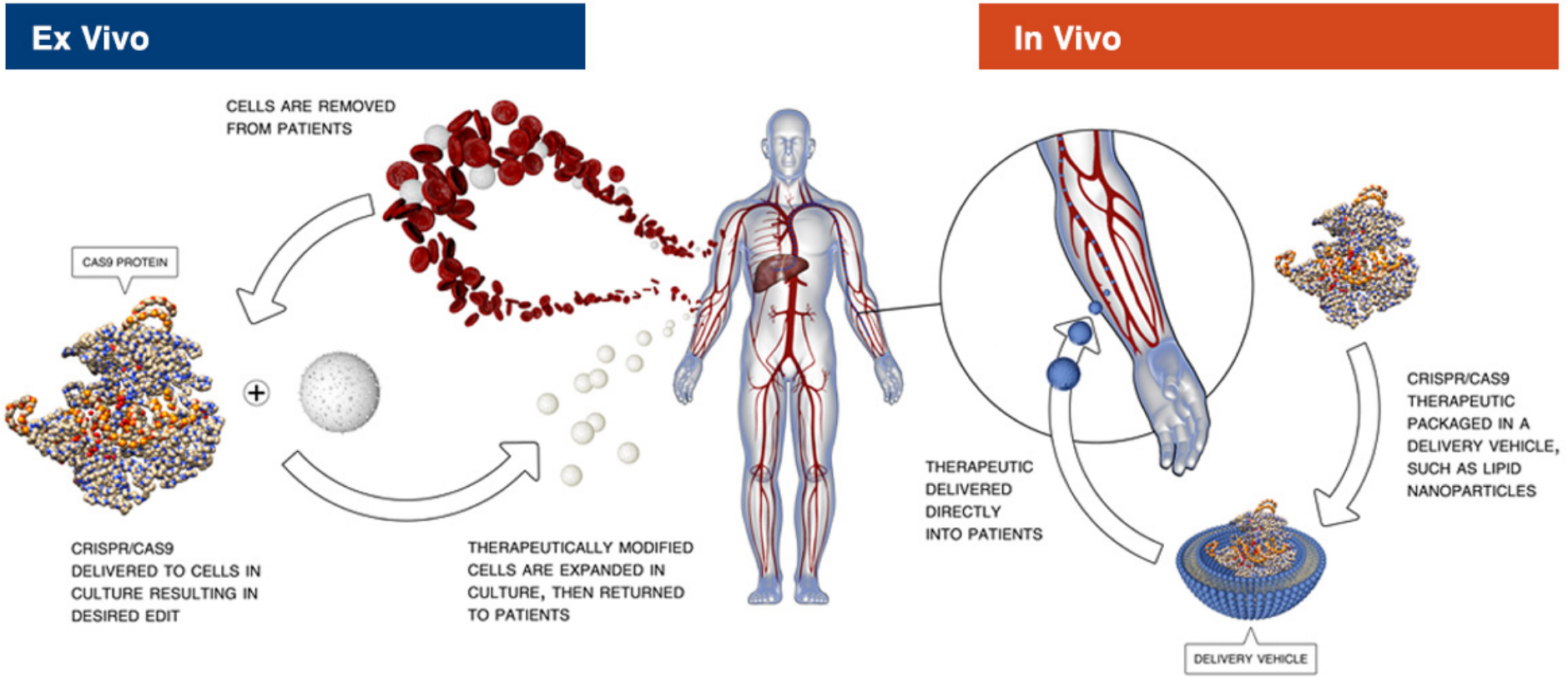
Received 22 September 2014 Accepted 31 March 2015 Published 14 May 2015

# Commercialization of CRISPR/CAS9 for Gene Therapy

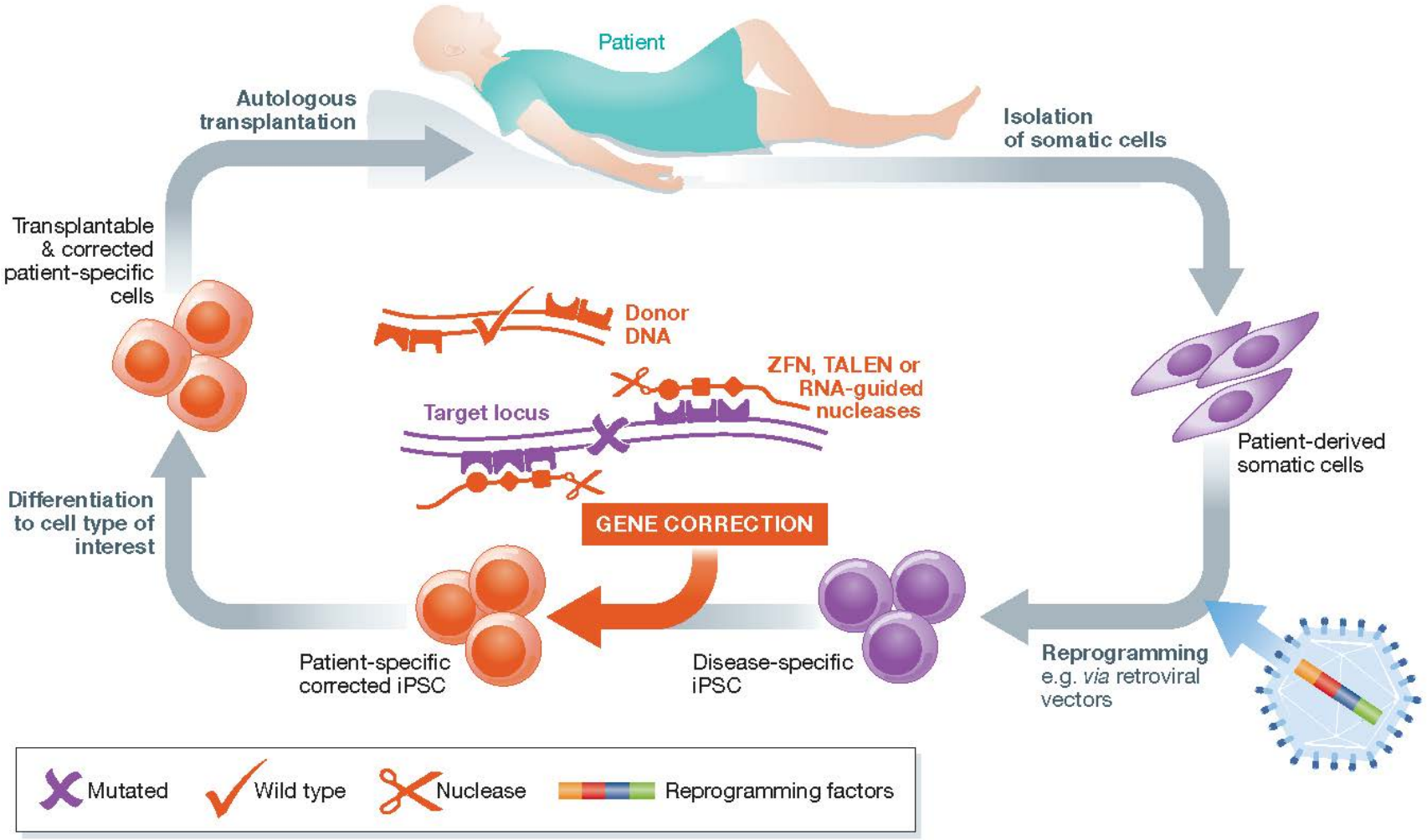
CRISPR THERAPEUTICS

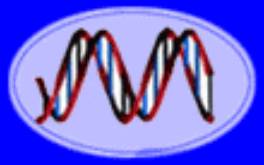
Intellia THERAPEUTICS

editas MEDICINE



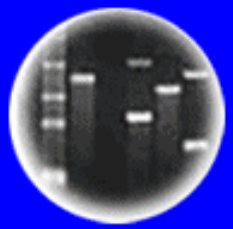
# Human Genome Editing Therapy





DNA
Genetic Code of Life

Entire Genetic Code of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

# The End!!

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