Gene Therapy

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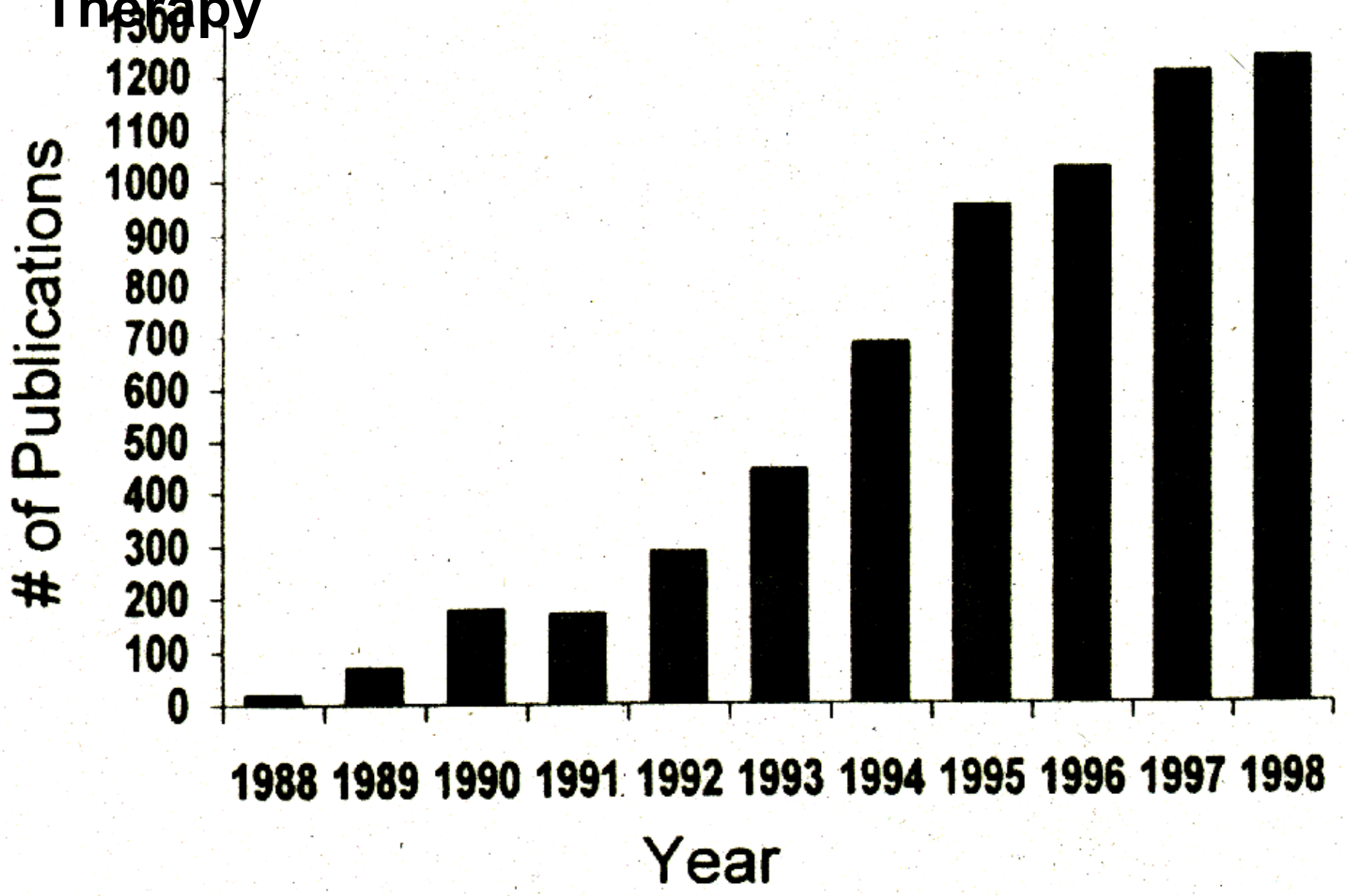
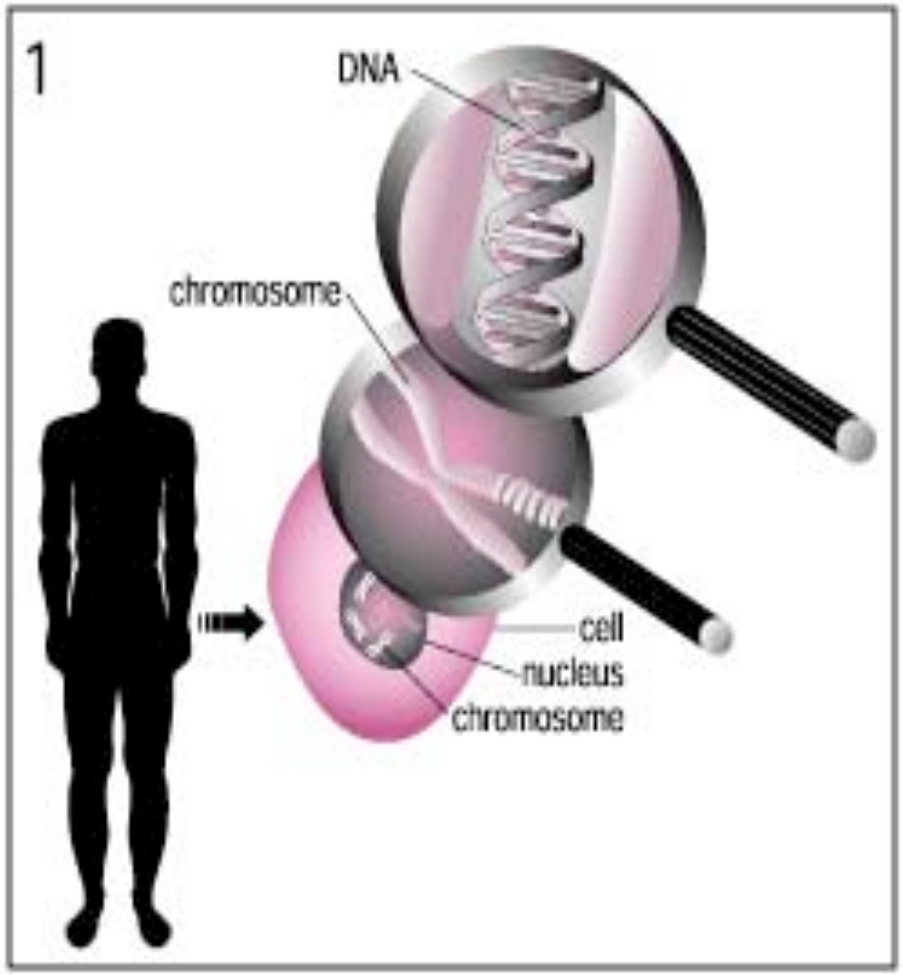
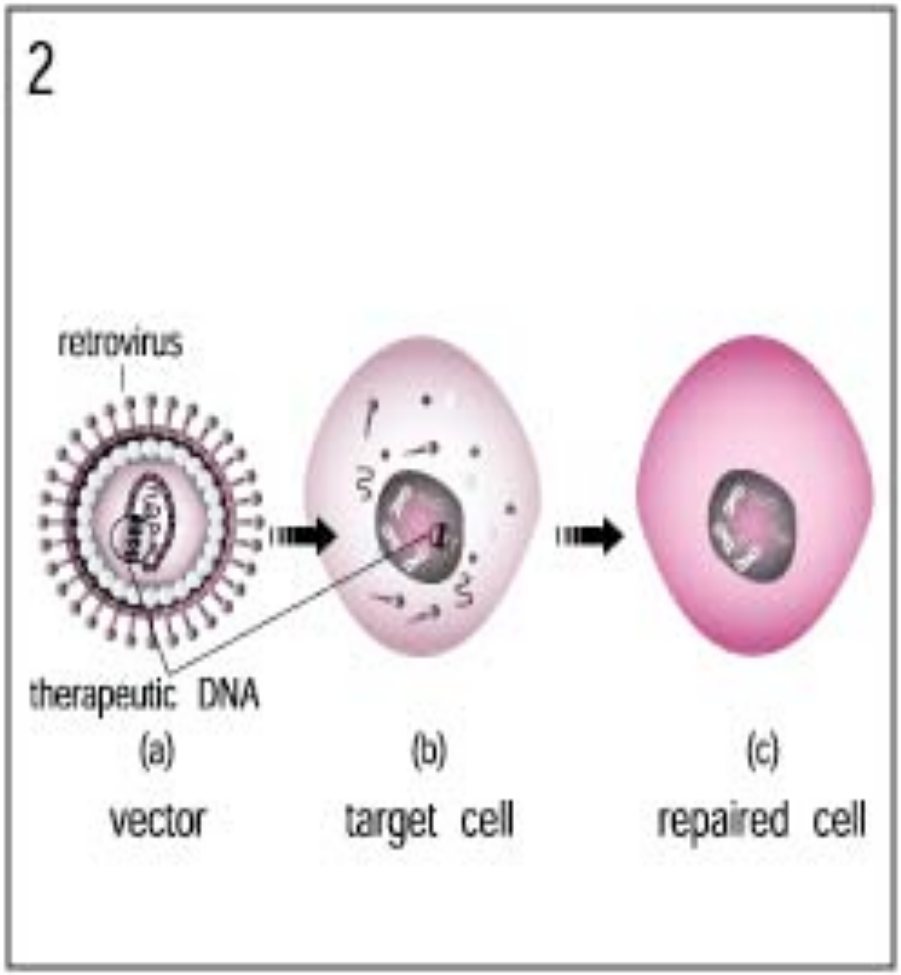
The Fascinating and Growing Field of Gene Therapy



DEFINITION OF GENE THERAPY

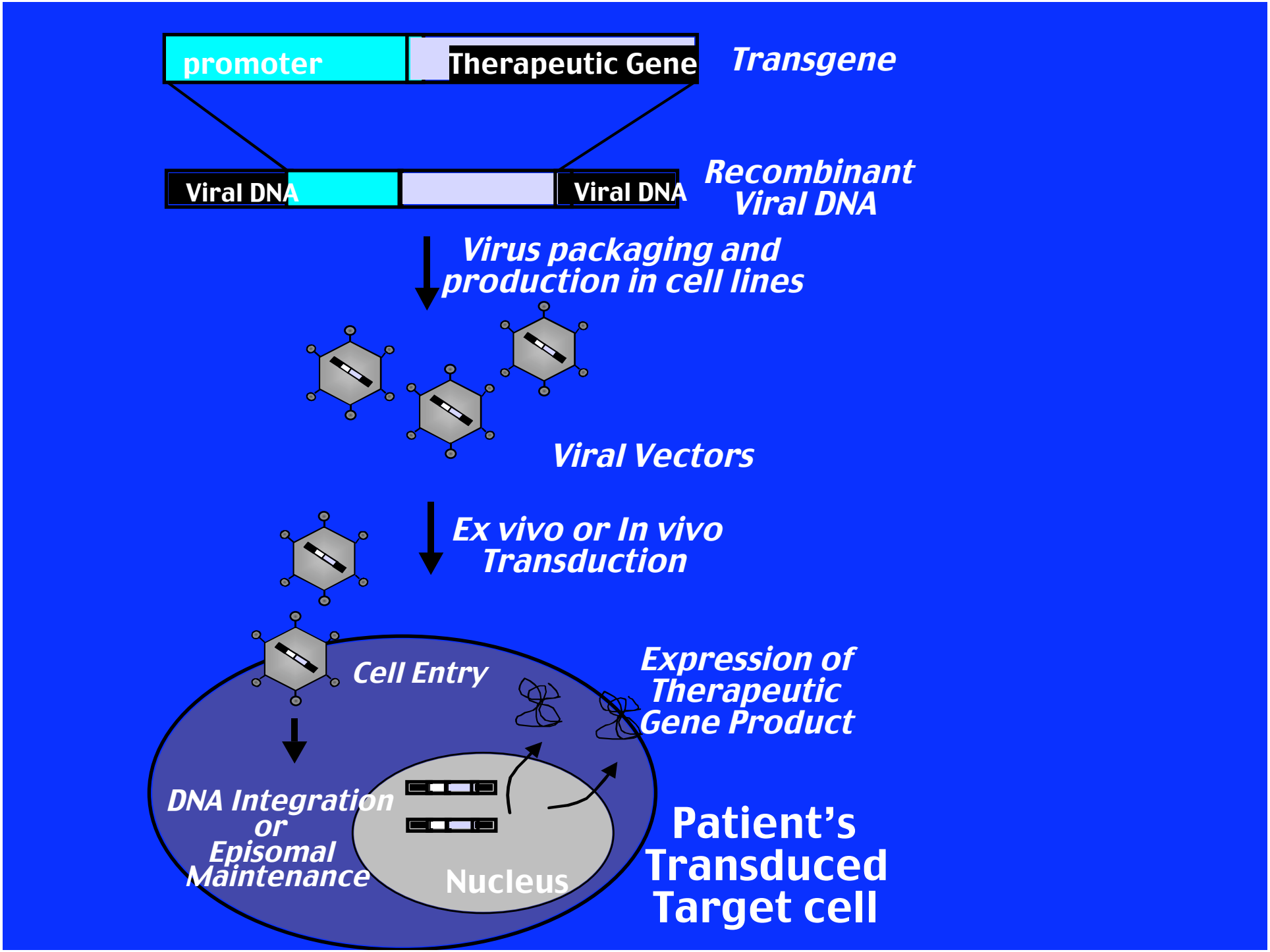
Gene therapy can be defined as the introduction of nucleic acids (e.g. DNA/genes) into cells of the body to correct or prevent a pathological process


1
DNA
chromosome
cell
nucleus
chromosome


2
retrovirus
therapeutic DNA
(a)
vector
(b)
target cell
(c)
repaired cell

What Do We Want to Accomplish?

- **Fix a gene-*mutation repair***
- **Add a gene-*restore a missing functi***
- **Silence a gene-*from a pathogen, gain of function mutation***


promoter
Therapeutic Gene
Transgene
Viral DNA
Viral DNA
Recombinant Viral DNA
Virus packaging and production in cell lines
Viral Vectors
Ex vivo or In vivo Transduction
Cell Entry
Expression of Therapeutic Gene Product
DNA Integration or Episomal Maintenance
Nucleus
Patient's Transduced Target cell

Early Gene Therapy Trials

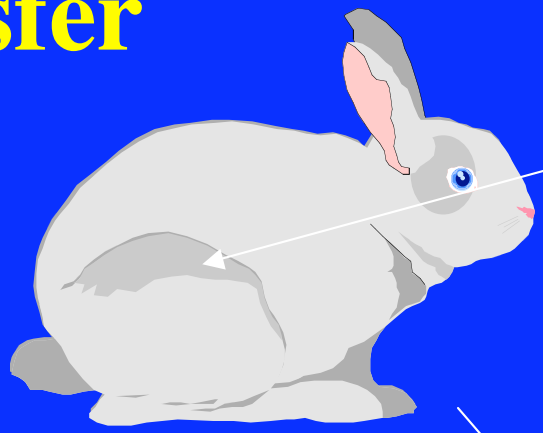
- Organ Transplantation
- 1975 Unsuccessful treatment of Arginase deficiency in Germany by infusing a rabbit virus into affected humans

DEFINITIONS

- **ExVivo- Remove cells from the body, genetic modification, and reintroduce the genetically modified cells**

- **In Vivo-Direct delivery of the therapeutic gene into the body**

Liver Directed Gene Transfer



In vivo

-Ferry et al., PNAS1991 88:8377
-Kaleko et al. Human Gene Ther 1991 1:27
-Kay et al., Hum Gene Ther 1992 3:641
-Kay et al 1993 Science

Partial hepatectomy

Ex Vivo

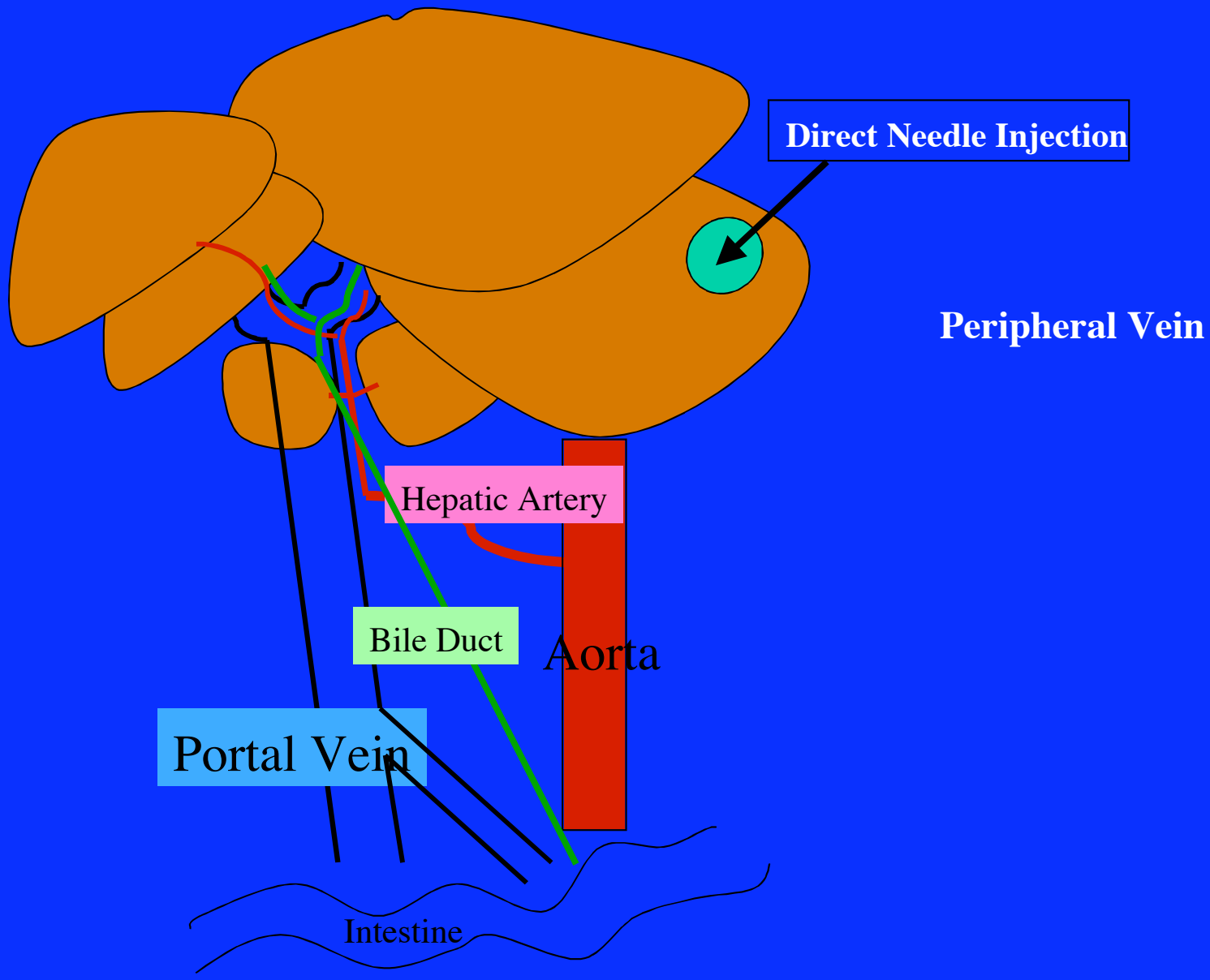
Transplantation of genetically modified autologous hepatocytes

-Chowdhury et al., Science 1991, 254:1802
-Kay et al., PNAS 1992.89:89

Transduce with integrating vector

Culture hepatocytes

Methods of In Vivo Vector Infusion



Gene Therapy

- The important tools for gene therapy include the development and testing of vectors which are vehicles that carry genes into our cells

- No vector is perfect

- Inherent properties of the vector and disease under study will require the development of different vector systems

Vector Categories

***Viral-Based*-Manipulating Natural Existing Viruses to Transfer Therapeutic Genes**

***Non-Viral*- DNA Molecules Delivered as Naked DNA, Lipids, or Polymers**

***Hybrid Vectors*- Some Combination of Different Viruses or Carriers**

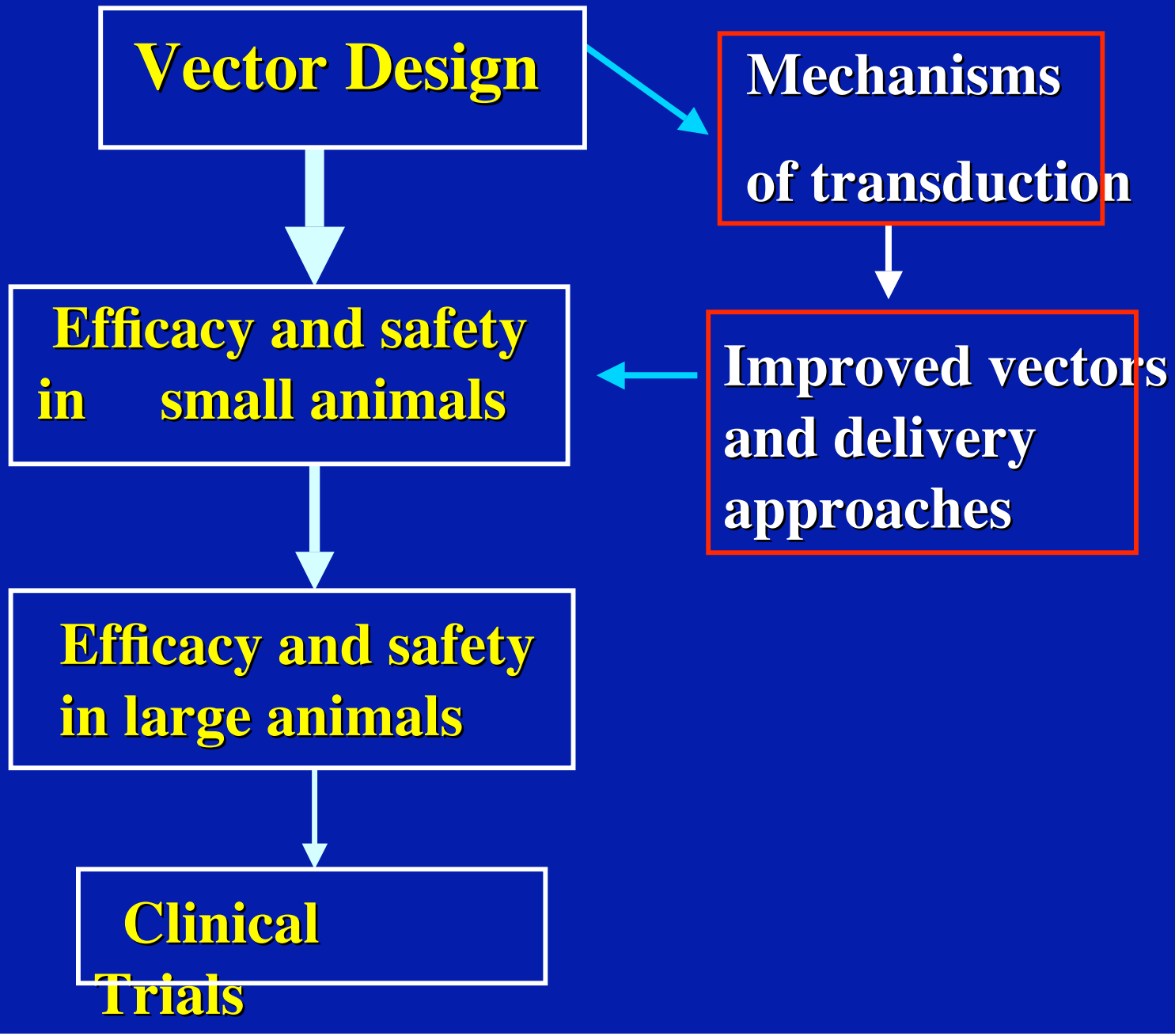
Examples of Viral Vectors

- Retroviruses/Lentiviruses-integrating vector
- Adenovirus-episomal
- Adeno-associated Virus-episomal/integrating?

Examples of Nonviral Vectors

- Naked DNA
- Liposomes
- Protein/lipid/CHO polymers

Bench to Bedside Approach



Levels of Review of Gene Therapy Protocols

University/Hospital:

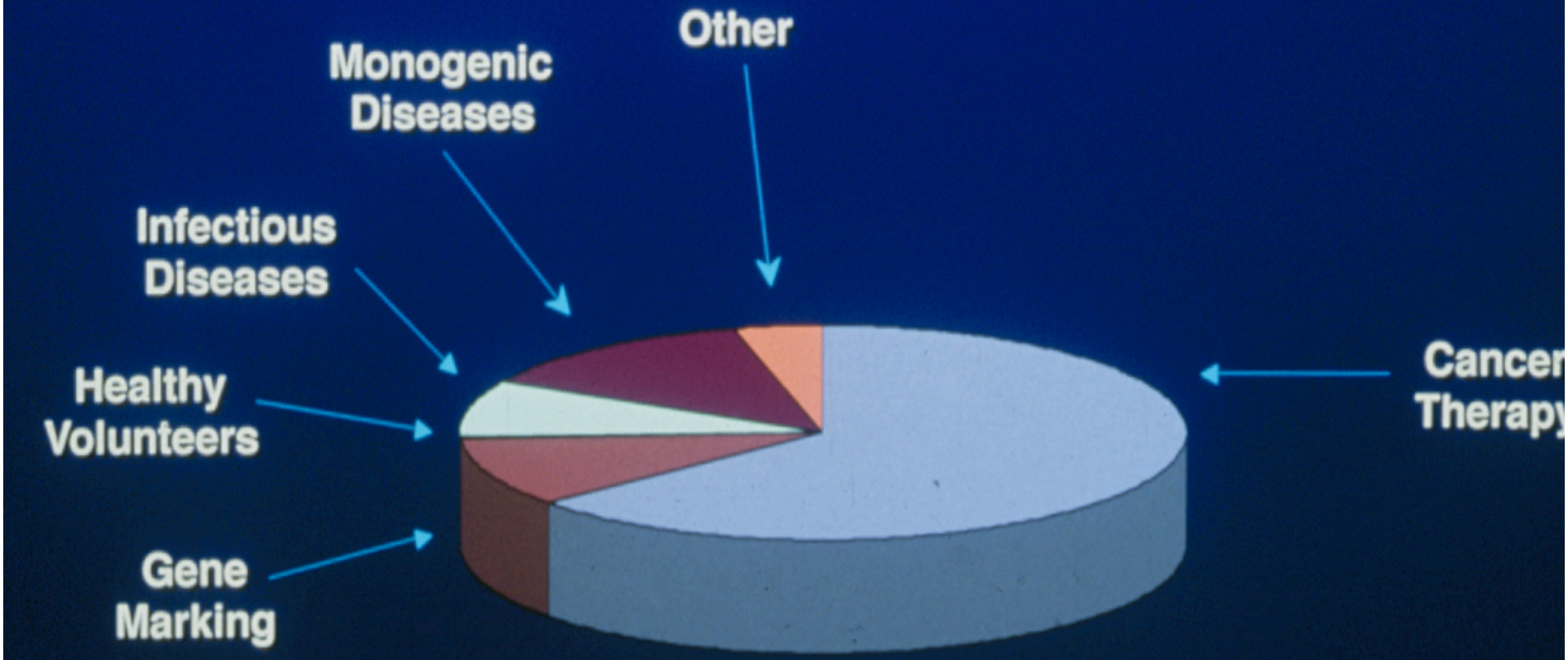
- Human subjects committee (IRB)
- Institutional Biosafety committee (IBC)

Federal:

- NIH Recombinant DNA Advisory Committee (RAC)
  (a.k.a Office of Biotechnology Assessment {OBA})
- Food and Drug Administration (FDA)

Candidate Diseases for Gene Therapy

Protocols by Disease



DISEASES AMENABLE TO GENE THERAPY-1

A. Genetic Disorders

1. Inborn errors of metabolism
2. Hemoglobinopathies
3. Coagulation disorders
4. Pulmonary diseases-CF, AAT deficiency
5. Hyperlipidemias
6. Muscular dystrophies

DISEASES AMENABLE TO GENE THERAPY-2

B. Acquired Diseases

1. Infections- (HIV, Hepatitis)

2. Neurological- (Parkinson)

3. Heart disease- (CAD, PVD, myocardial infarct)

4. Musculoskeletal-arthritis

5. Endocrine-Diabetes

6. Vaccines

DISEASES AMENABLE TO GENE THERAPY-3

B. Acquired Diseases (continued)

6. Cancer

i) Immune based-vaccine,antigen, cytokine

ii) Cellular toxins- prodrug conversion

iii) Gene regulation- p53, antisense

iv) Replicating lytic vectors

GENE THERAPY FOR GENETIC DISEASES-*loss of function*

- **PERSISTENCE OF GENE EXPRESSION**
  - **-regulated vs constitutive**
- **STEM CELLS vs "PERMANENT" CELLS**
  - **-bone marrow, skin, CNS, liver**
- **EPISOMAL VS INTEGRATING VECTORS**

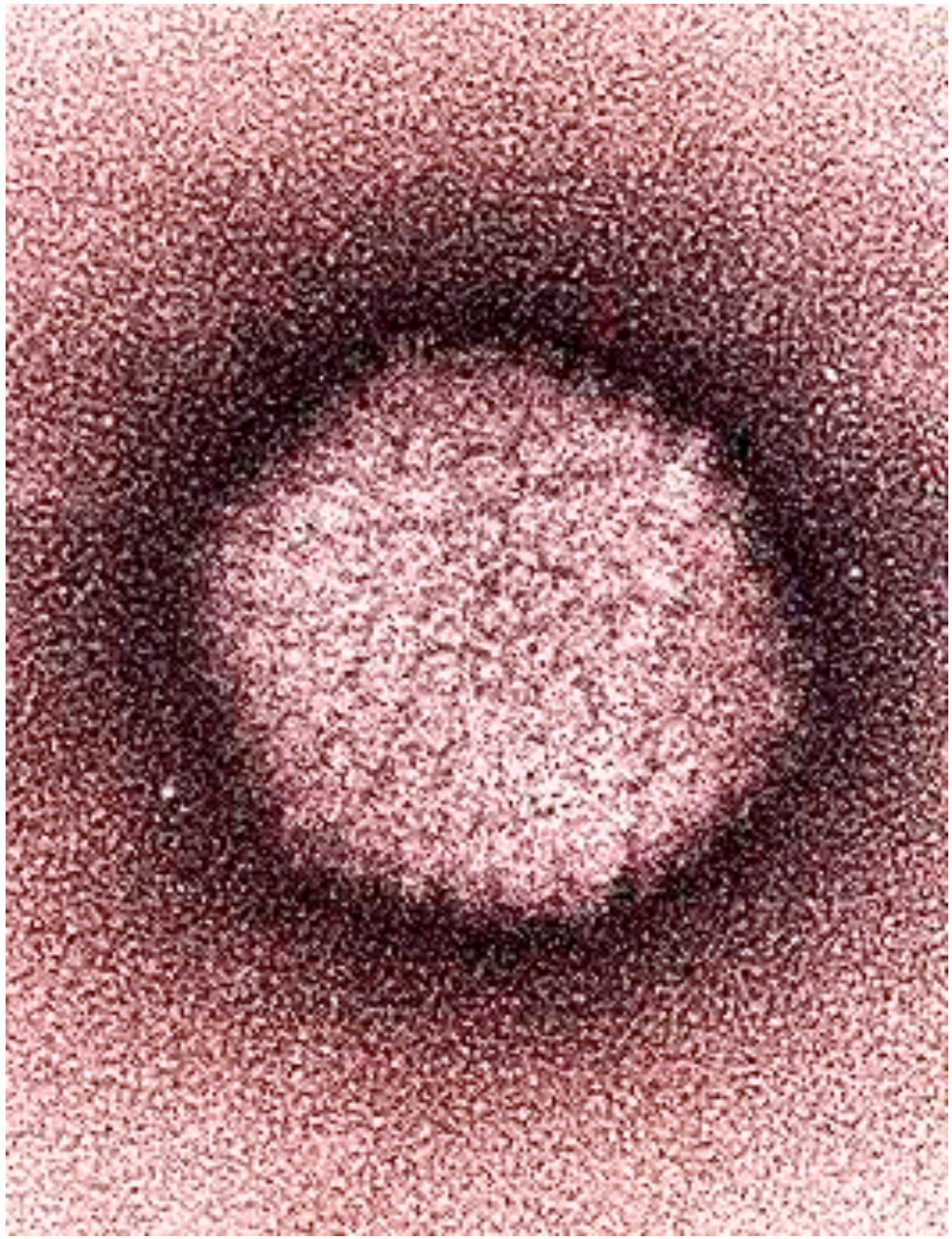
Recombinant Retroviruses

Most Used Vector in Gene Therapy

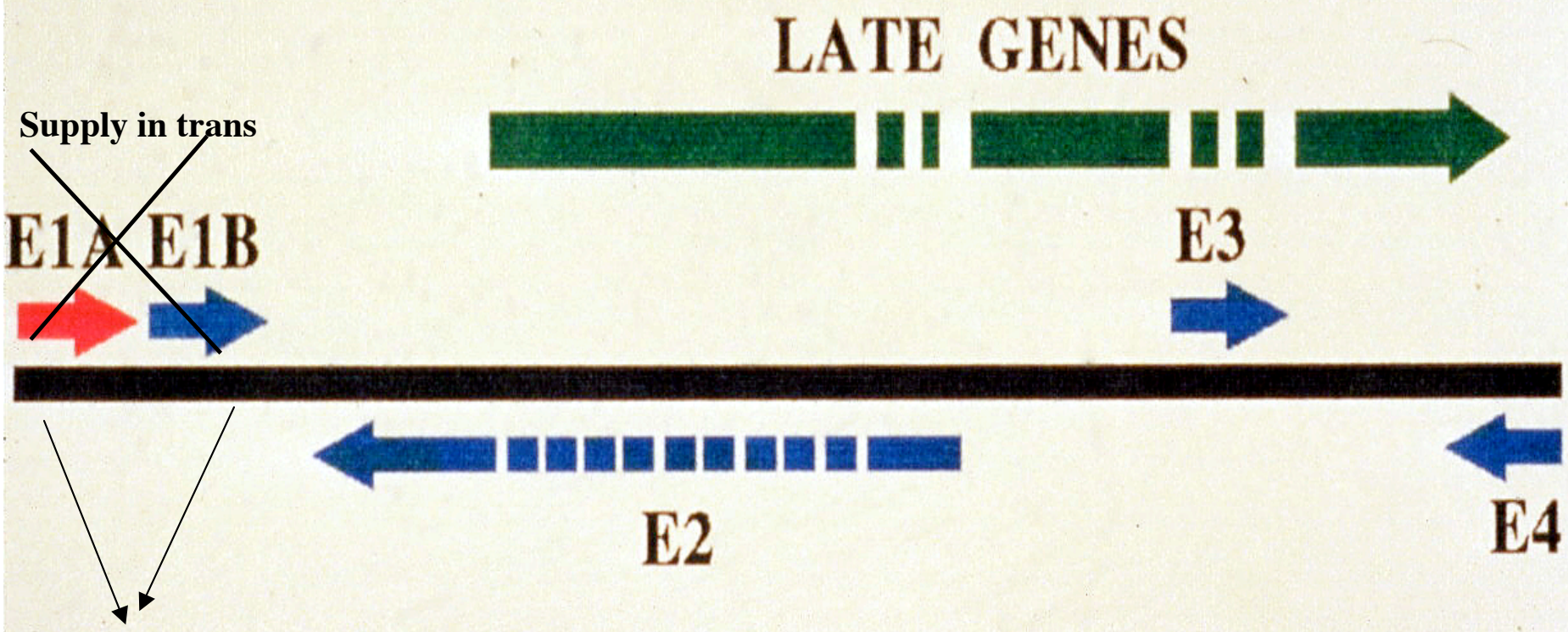
- **Relatively Safe and can be produced in packaging cells**
- **Integrating Vector - but only into dividing cells**

Different Varieties

- **Mouse Moloney-Oncoretrovirus**
- **Lentivirus-derived from HIV**
- **Foamy?**

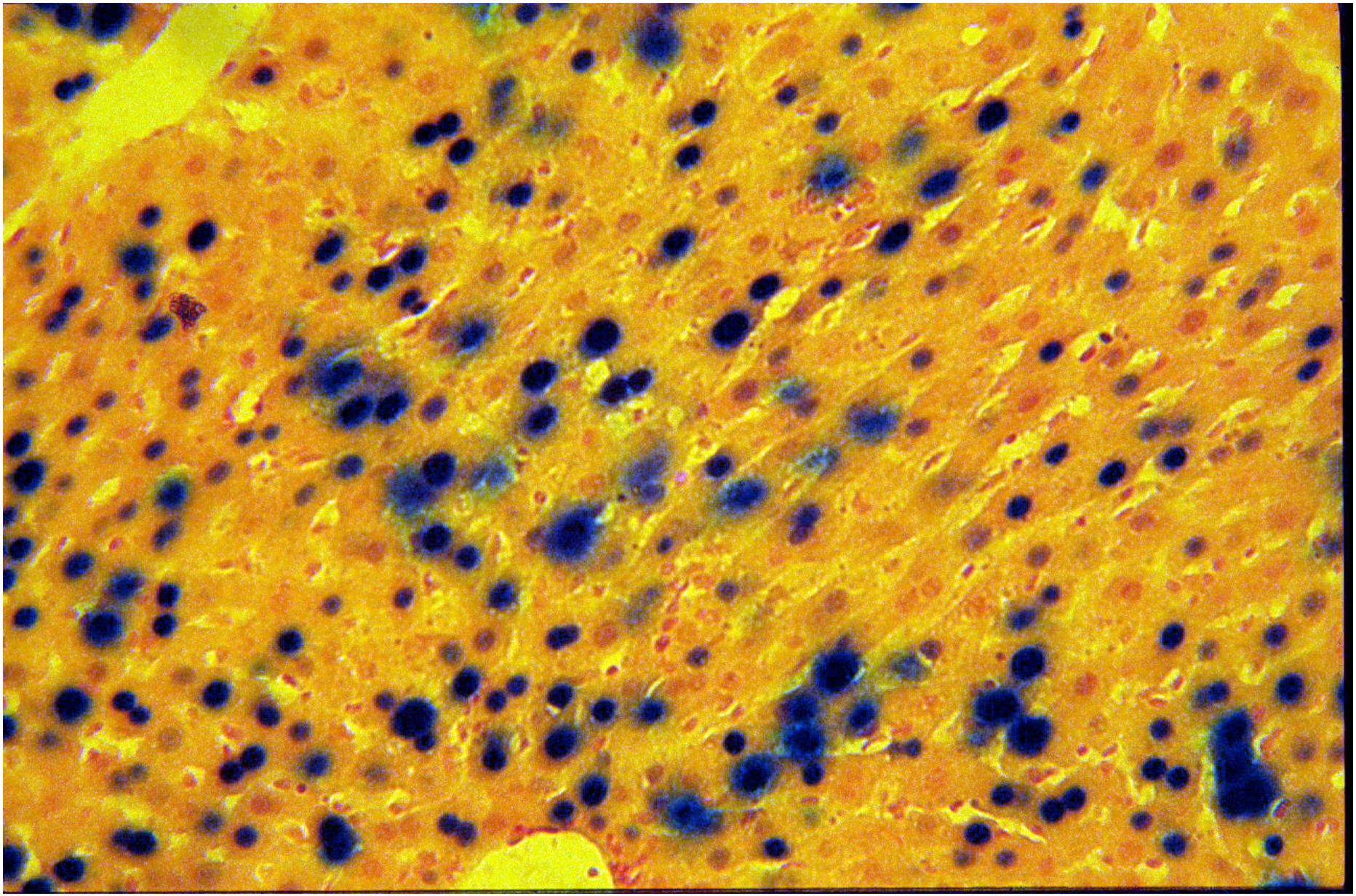


ADENOVIRUS GENOME



Expression cassette-therapeutic gene

EARLY ADENOVIRAL TRANSDUCTION STUDIES



Early Adenoviruses

- First, Second, and Third Generation vectors-relatively high titers
- All of these vectors contain some viral genes
- Acute toxicity
- Persistent cytotoxicity and immunogenicity

General Issues Regarding Adenoviral Vectors

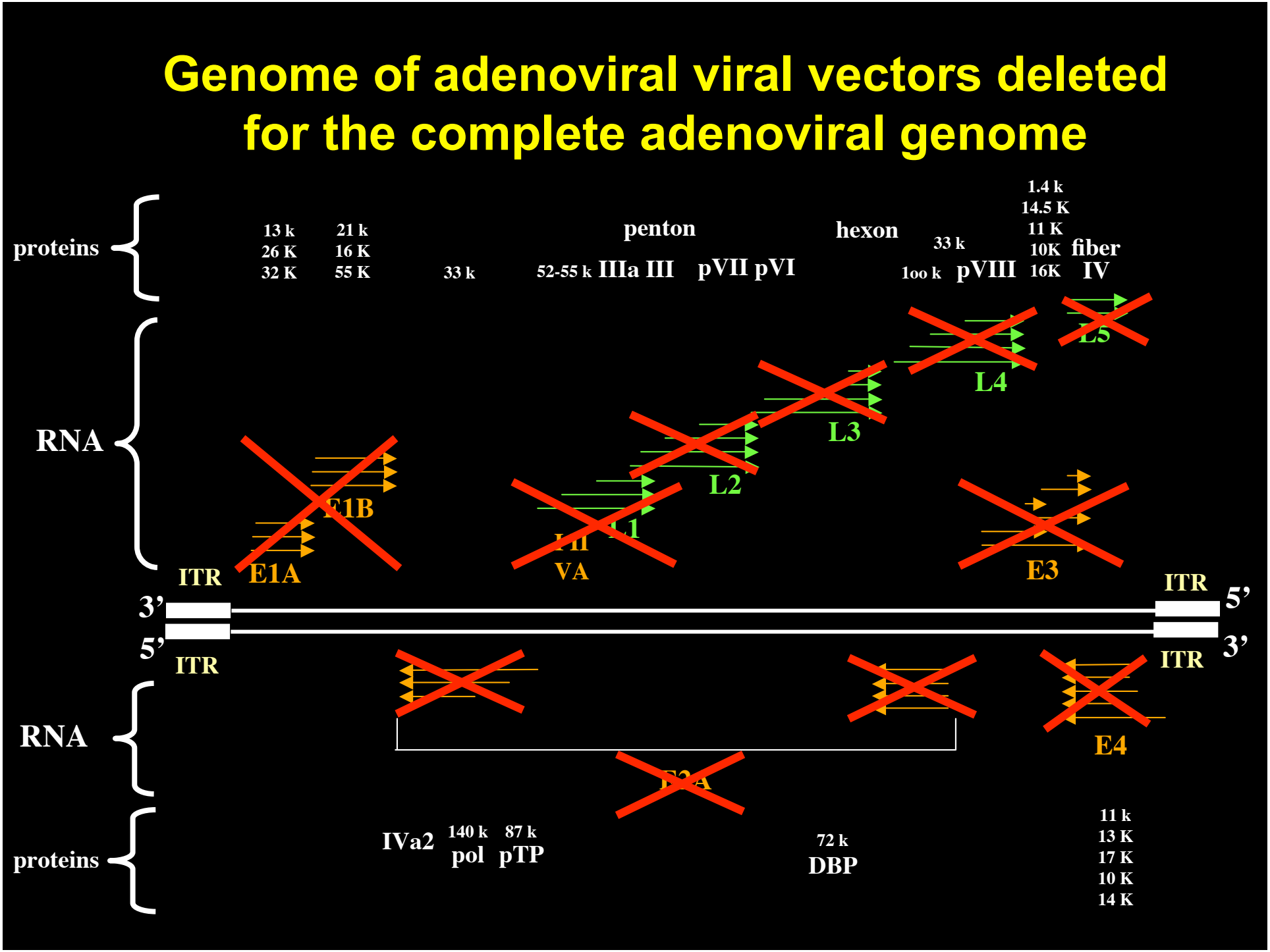
- Most efficient vector for hepatic gene transfer reaching 100% hepatocyte transduction
- Can transduce many but not all cell types
- The vector does not integrate

A PHASE I STUDY OF ADENOVIRAL VECTOR MEDIATED GENE TRANSFER TO LIVER IN ADULTS WITH PARTIAL ORNITHINE TRANSCARBAMYLASE DEFICIENCY (OTC)

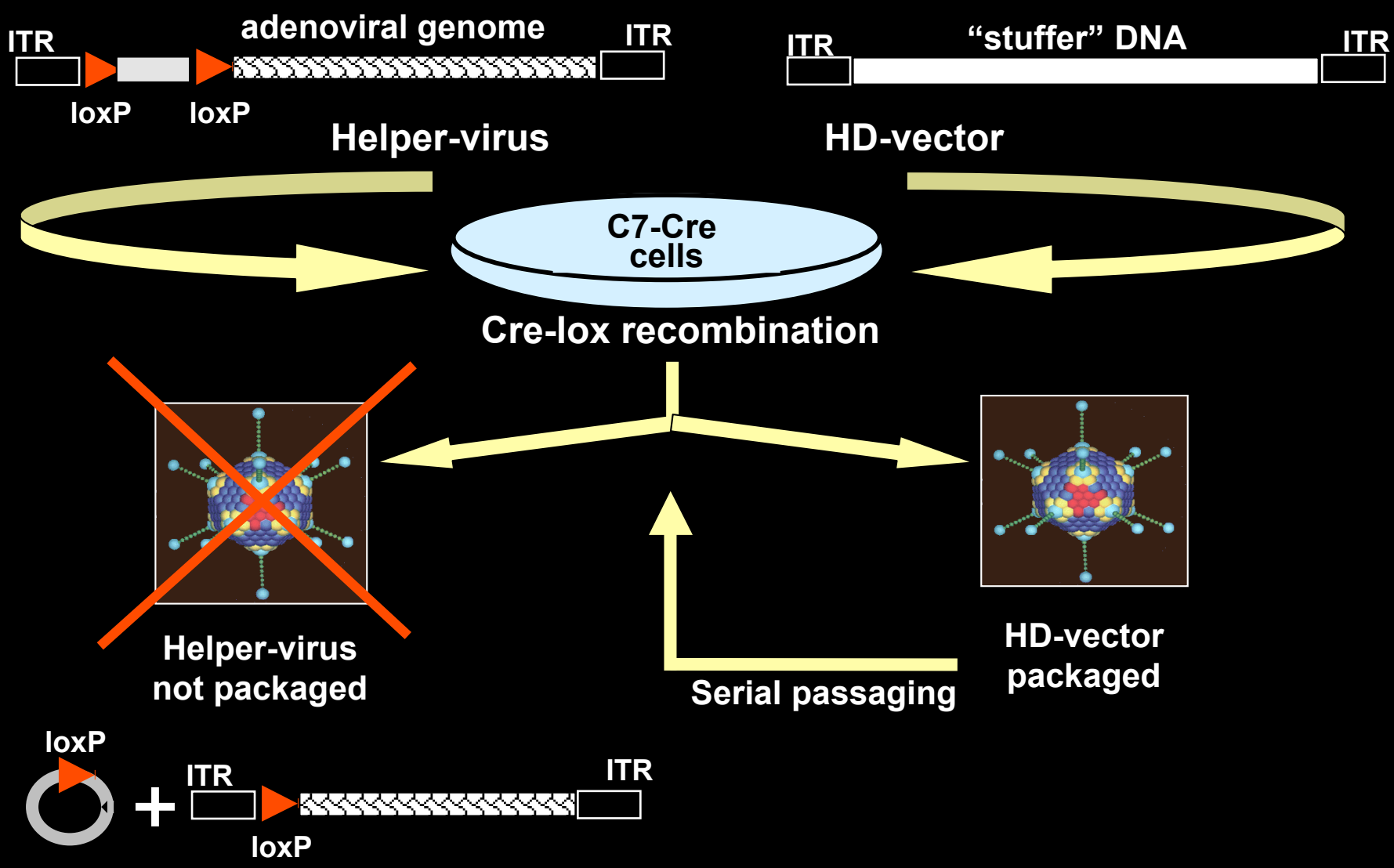
18 y/o male with moderate form of OTC deficiency volunteered for study.

He received an E1/E4-deleted, type 5 adenoviral vector carrying the human OTC cDNA driven by a CMV enhancer/chicken beta-actin promoter by intrahepatic artery injection of 3x10e13 p.f.u. (2nd patient to receive this dosage).

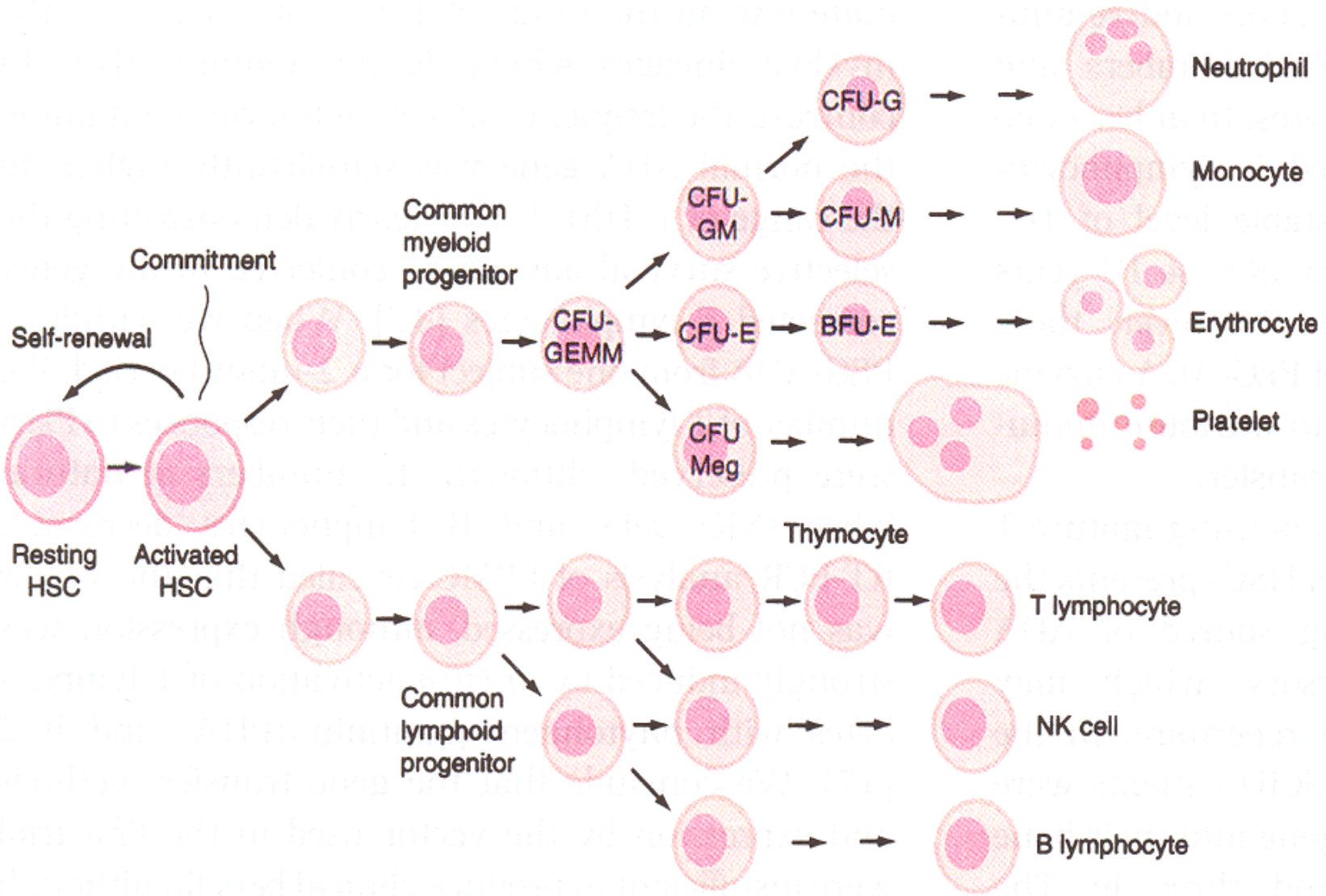
Genome of adenoviral viral vectors deleted for the complete adenoviral genome



Production of gutless adenoviruses



Hematopoietic Stem Cell Differentiation



Severe Combined Immune Deficiency (SCID)

SCID is the genetic absence of a protective immune system.

Usually absent T cells and absent or non-functioning B cells.

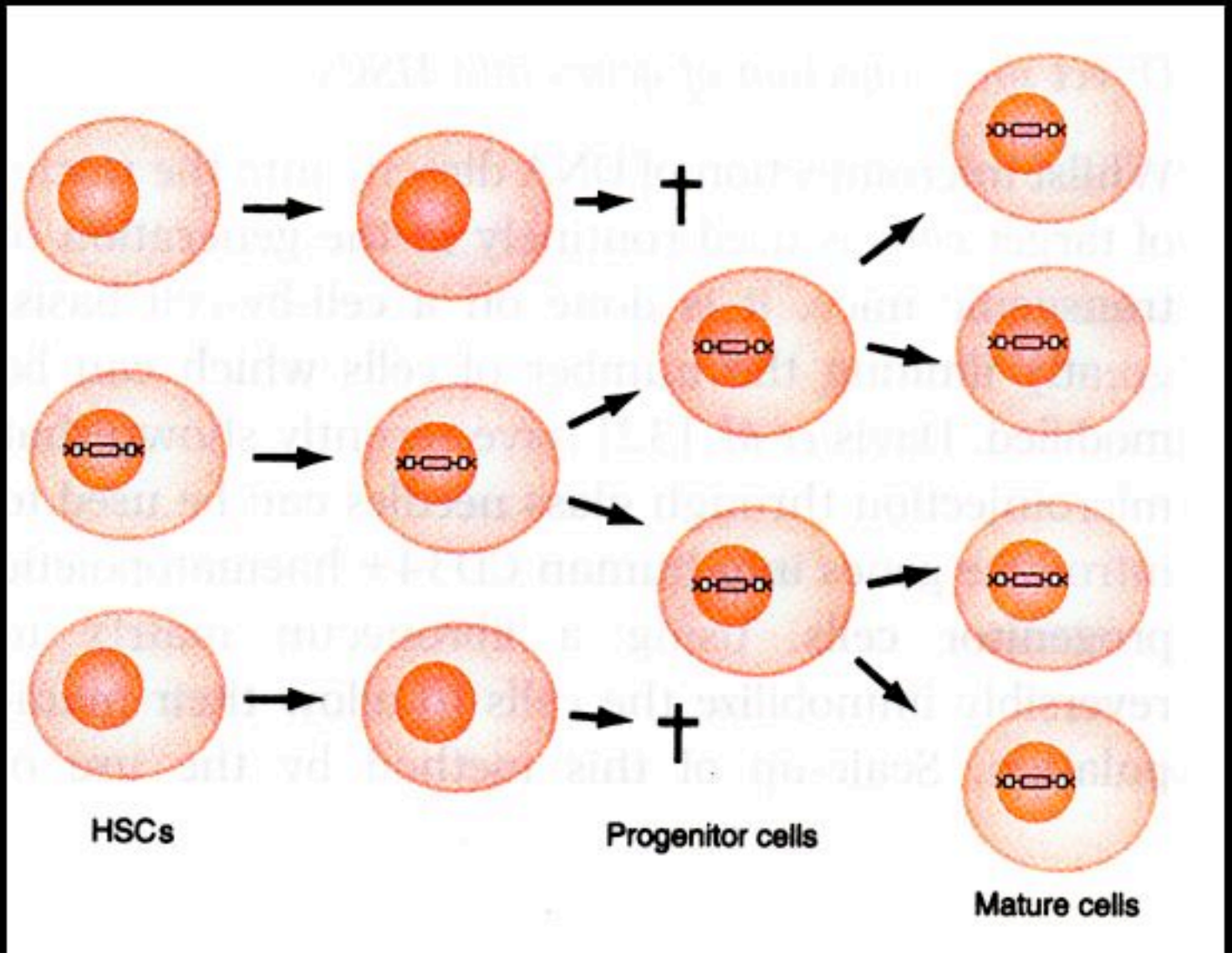
Can be caused by defects in a number of different genes:

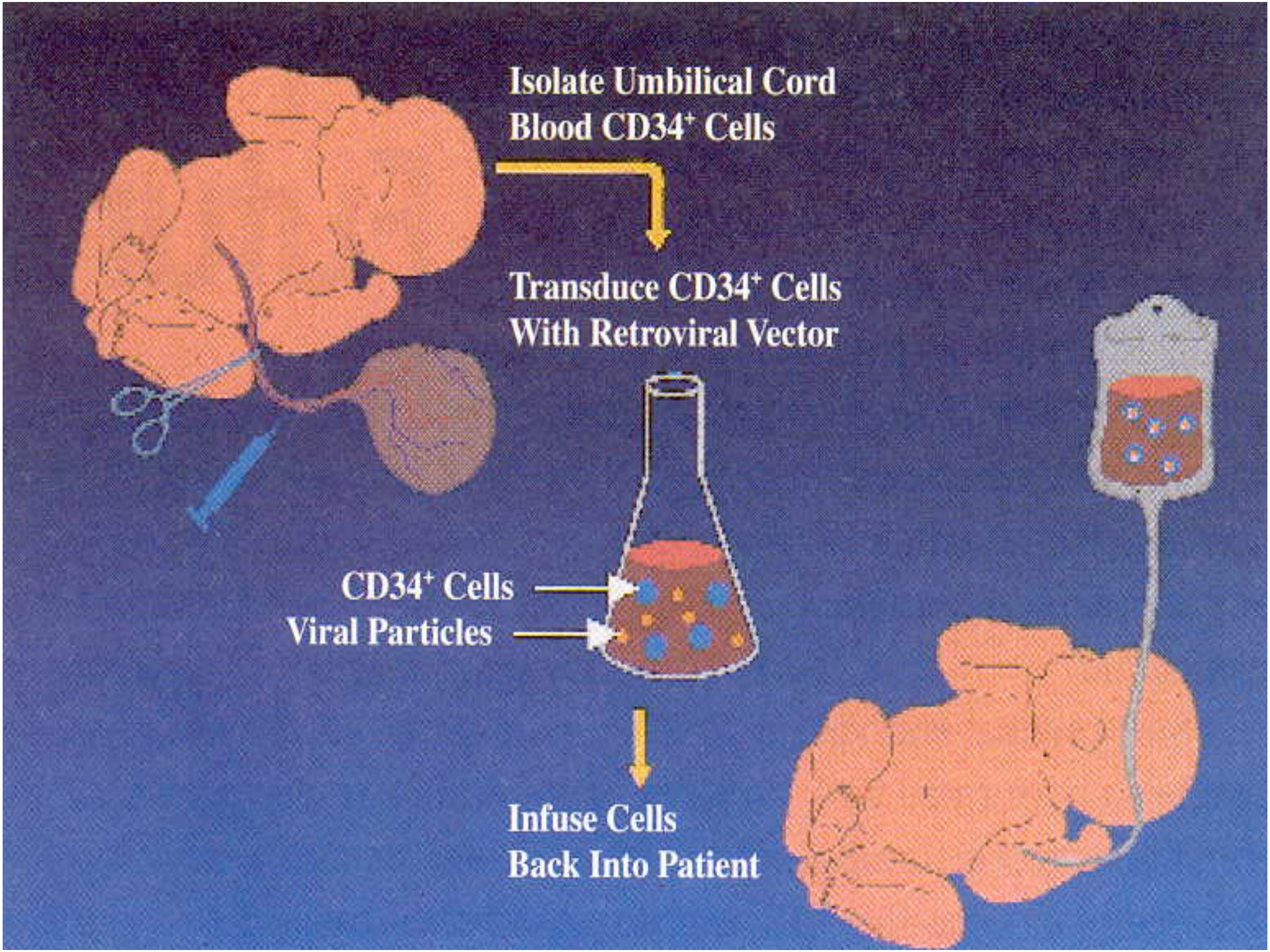
adenosine deaminase (ADA), common cytokine receptor,

Can be cured by BMT from an HLA-matched sibling; BMT from less well-matched donor has lower success rate.

“Best-case scenario” for gene therapy using HSC because a complete immune system may develop from just a few gene-corrected HSC.

Selective Expansion from Gene-transduced HSC




Isolate Umbilical Cord
Blood CD34+ Cells
Transduce CD34+ Cells
With Retroviral Vector
CD34+ Cells
Viral Particles
Infuse Cells
Back Into Patient

French Study of X-linked SCID

First Clear Demonstration of Efficacy Due to Gene Therapy

Reconstitution of Immune Functio

BOTTOM LINE-IT WORKED!!

But……

Insertional Mutagenesis?

- 3 of 9 patients have developed leukemia
- Two have retroviral insertion in LMO-2
- Is this disease specific?

HEMOPHILIA

X-Linked Disorder (Deficiency of FVIII or FIX)

Small (mouse) and Large (Canine) Animal Models

Pathophysiology is well understood-clinical endpoints

Bleeding Diathesis:
Severe (<0.1%), Moderate (1-5%), and Mild (5-20%)

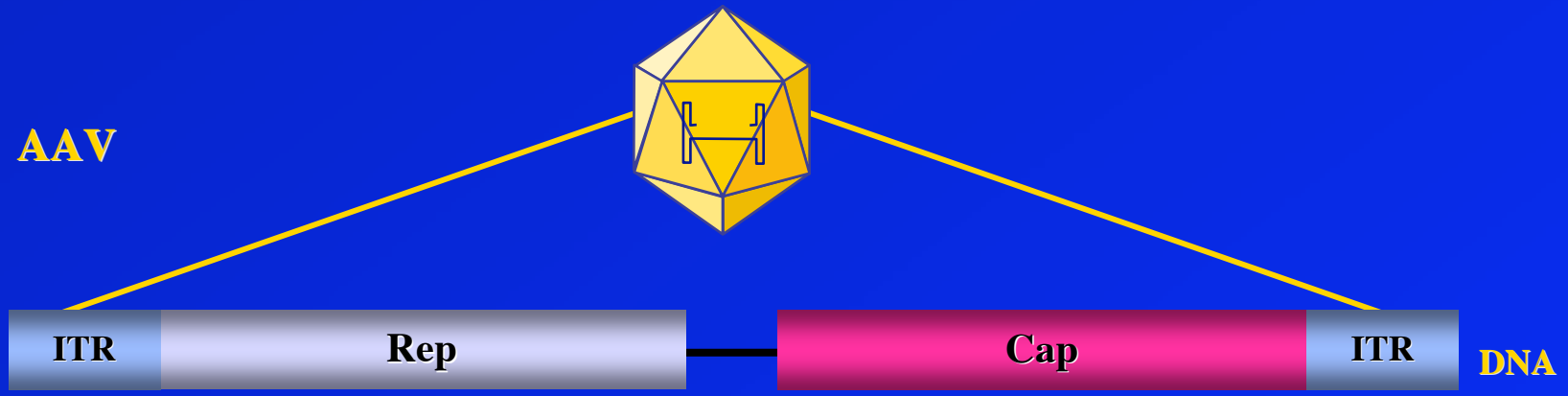
Phenotype is well correlated with plasma factor levels

Current Therapy consists of infusion of factor

Adenoassociated virus vectors

- Based on a non-pathogenic parvovirus
- All viral genes removed
- Outstanding Safety Profile
- Limited coding capacity
- Eight serotypes under study
- Muscle, Lung, Liver clinical trial started

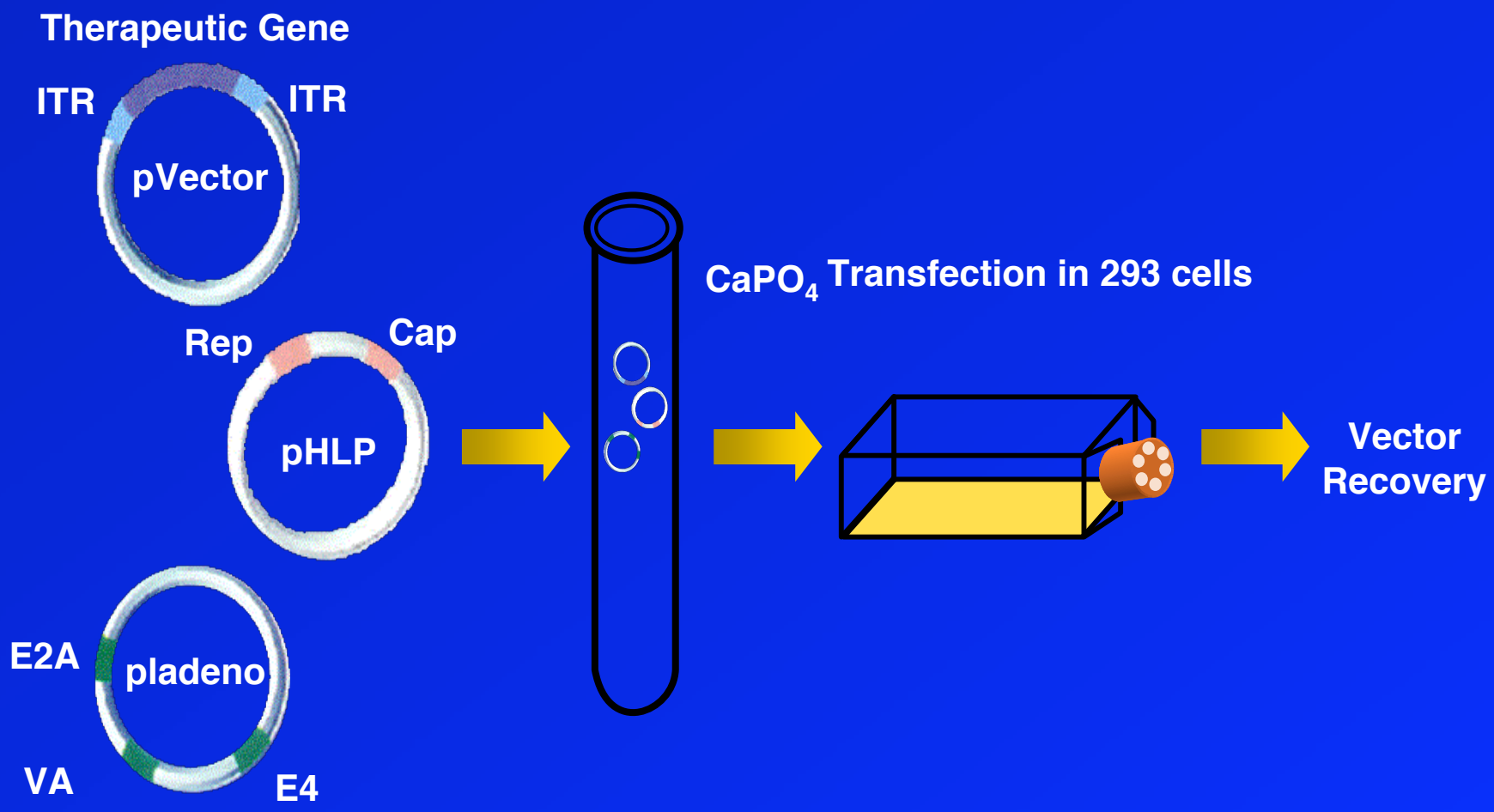
AAV Vectors


AAV
ITR
Rep
Cap
ITR
DNA


AAV Vectors
Gene Cassette
ITR
Control element
Therapeutic gene
ITR
DNA

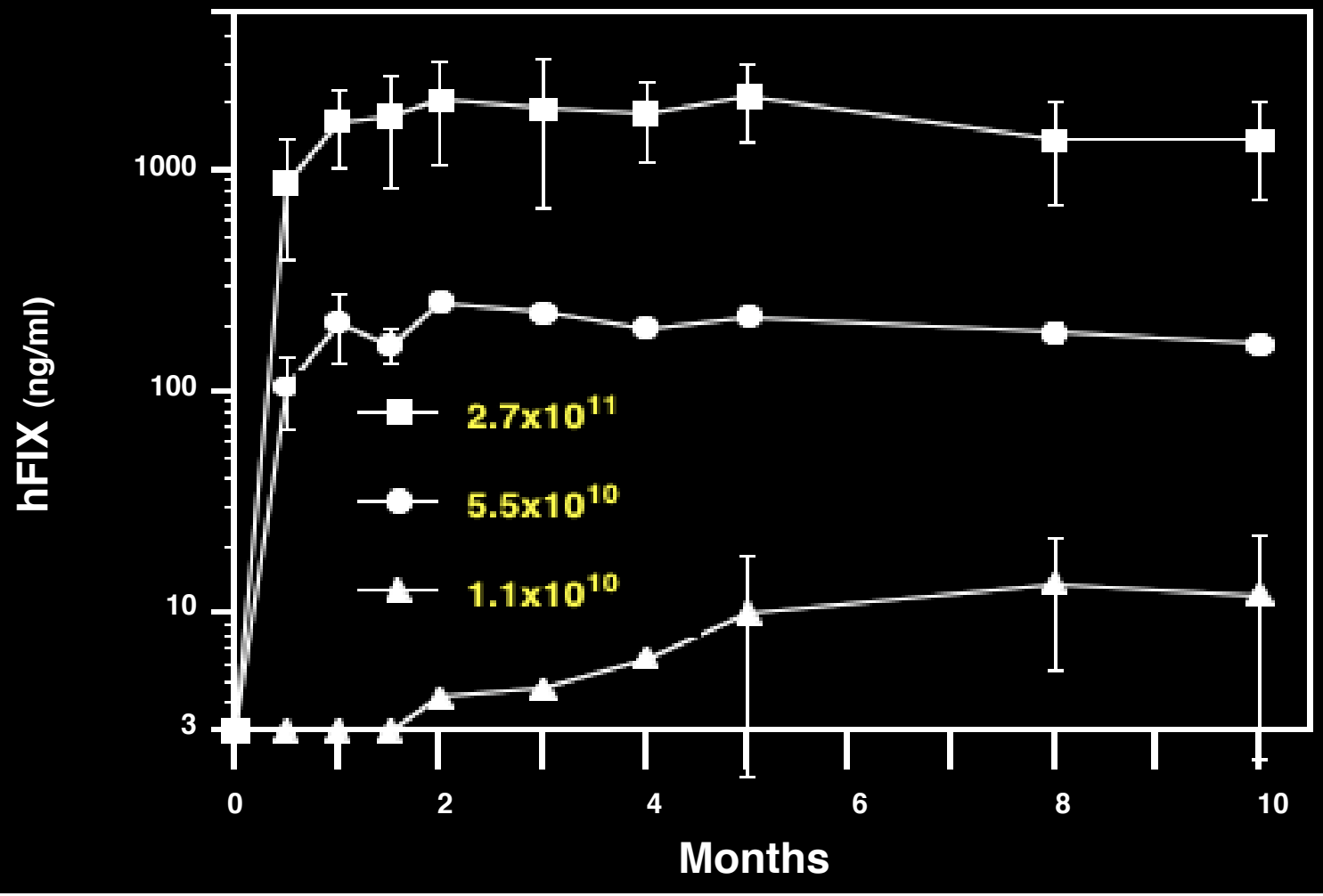
AAV Vector Production Strategies

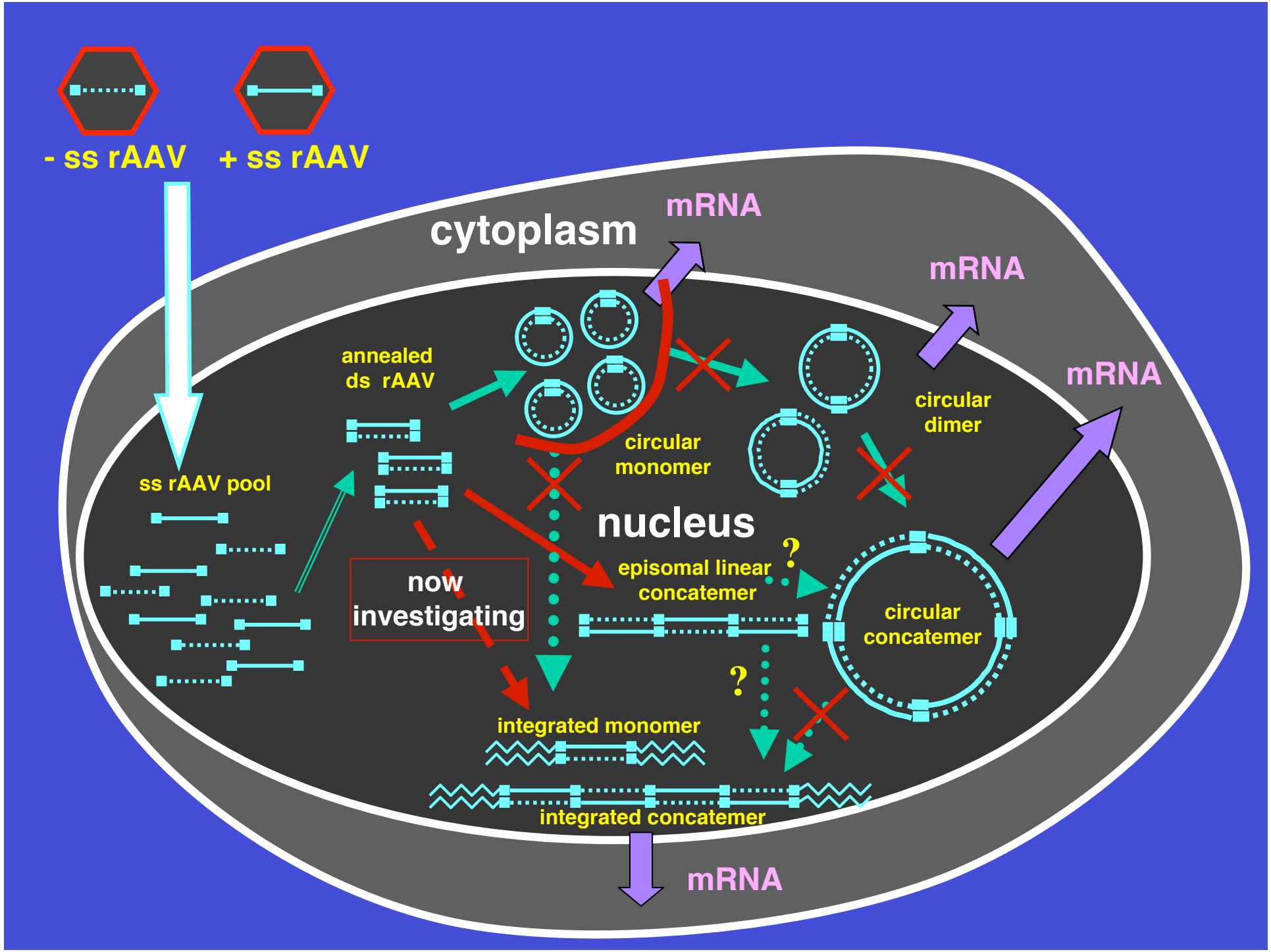
- Helper virus-free system



rAAV-mediated transgene expression *in vivo*

AAV-EF1α-hFIX to C57BL/6 mice




- ss rAAV
+ ss rAAV
cytoplasm
mRNA
mRNA
mRNA
annealed
ds rAAV
circular
monomer
circular
dimer
ss rAAV pool
nucleus
now
investigating
episomal linear
concatemer
?
circular
concatemer
?
integrated monomer
integrated concatemer
mRNA

Dogs Are Treated

- Dog colonies at University of North Carolina-Chapel Hill
- Dogs treated with AAV-FIX vectors
- Expression for 5 years

Well recovered. This hemophilia B dog, shown with technician Pamela McElveen, had factor IX gene therapy almost 9 months ago.

Liver-Derived Expression of cFIX in Hemophilia B Dogs Infused with ~1x10^{12} AAV-(ApoE)4/hAAT-cFIX/kg

AAV TOXICITY IN VIVO

- **Acute Toxicity-mice,rats,dogs, non-human primates**
- **Biodistribution-mice and rats**
- **Germ line Transmission-dogs**
- **Long-term toxicity-mice and dogs**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STOP
CLR
BOLUS
EDIT
LOCK
CONFIRM

Fixing Broken Genes

Gene Repair

Small DNA or DNA/RNA molecules can be made that are complementary to a segment of a gene, but which differ in 1 or 2 base pairs. These synthetic oligonucleotides can lead to repair or correction of a gene mutation.

e.g. Sickle cell disease results from a change of a single base-pair in the beta-globin gene.

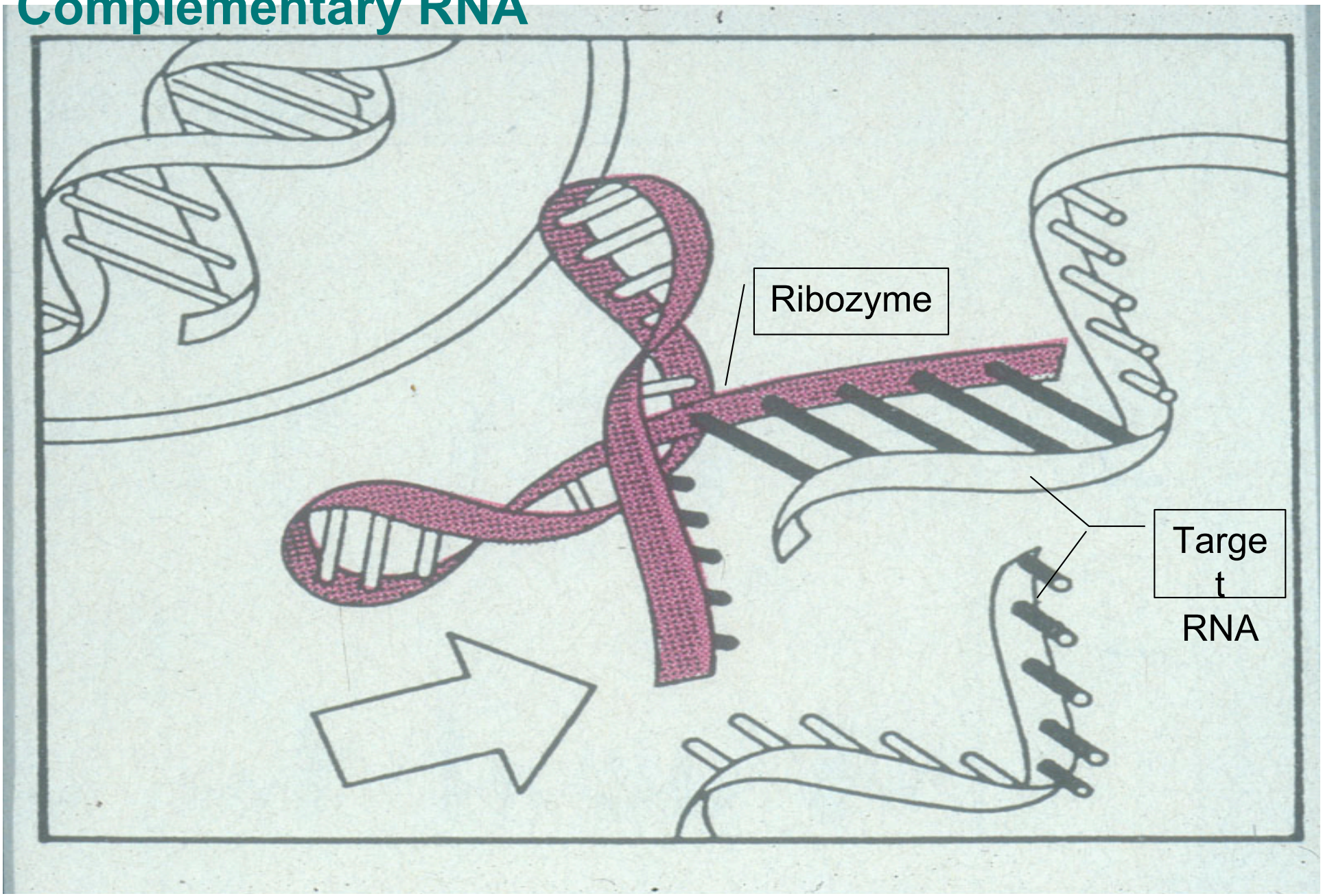
	Nl	HbS
Codon #6	GAA ->	GTA
A.A #6	Glu ->	Val

New Approach For Achieving Gene Knockdown

Post-Transcriptional Gene Silencing-PTGS

- *Ribozymes*
- *DNAzymes*
- *Anti-Sense*
- *Morpholinos*
- *RNAi*

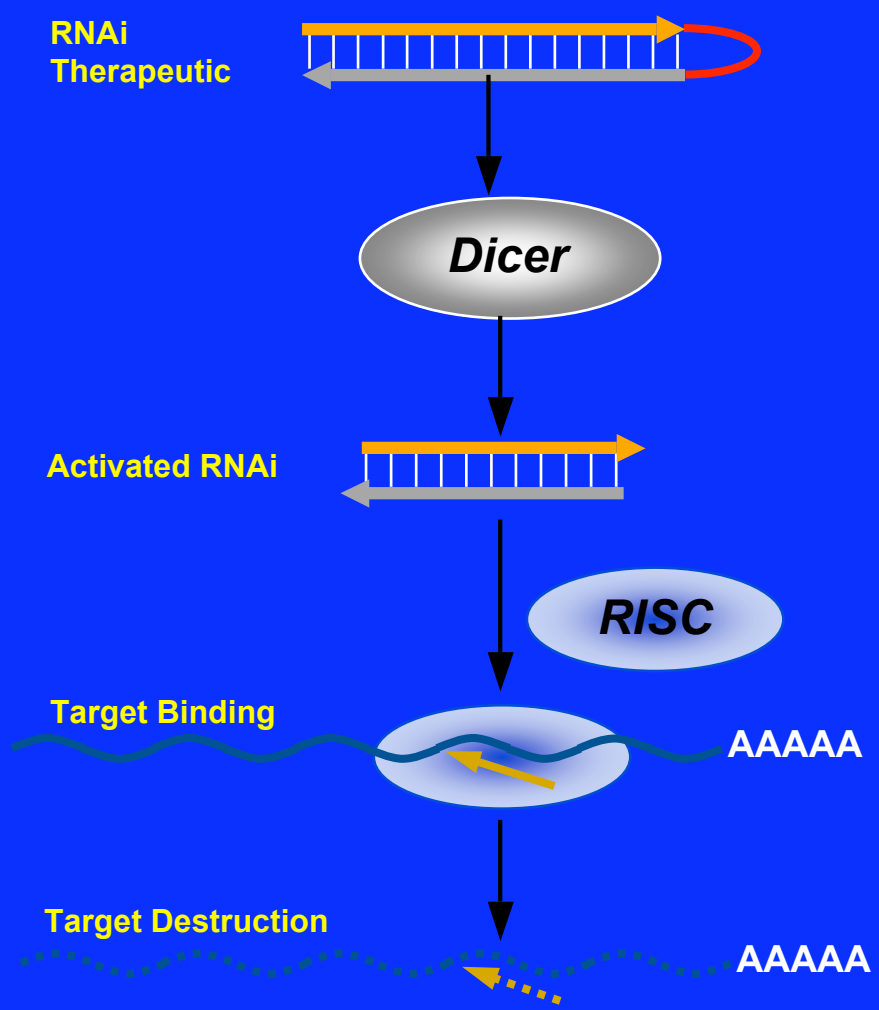
Ribozymes are RNA Enzymes that Cut Complementary RNA



RNA Interference (RNAi)

Defined as the process whereby double-stranded RNA induces sequence-dependent (based on homology) degradation of cognate mRNA

RNA interference (RNAi)

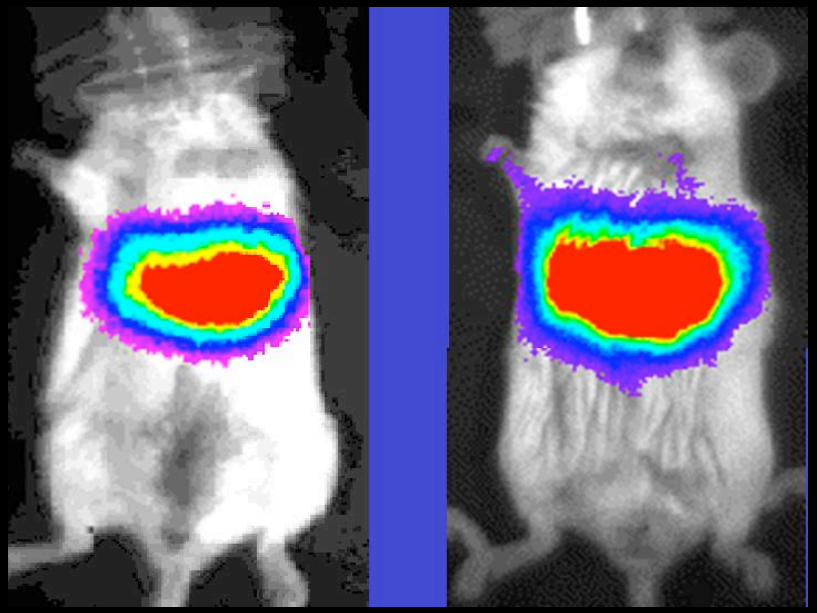


- Natural Mechanism
- Highly Specific

Gene Silencing Can Be Used To

-inhibit pathogenic micro-organism
from replicating in the host
-shutoff gain-of-function mutations
that cause genetic disease or canc

Luciferase RNA/DNA is transferred by hydrodynamic transfection to the livers of living mice



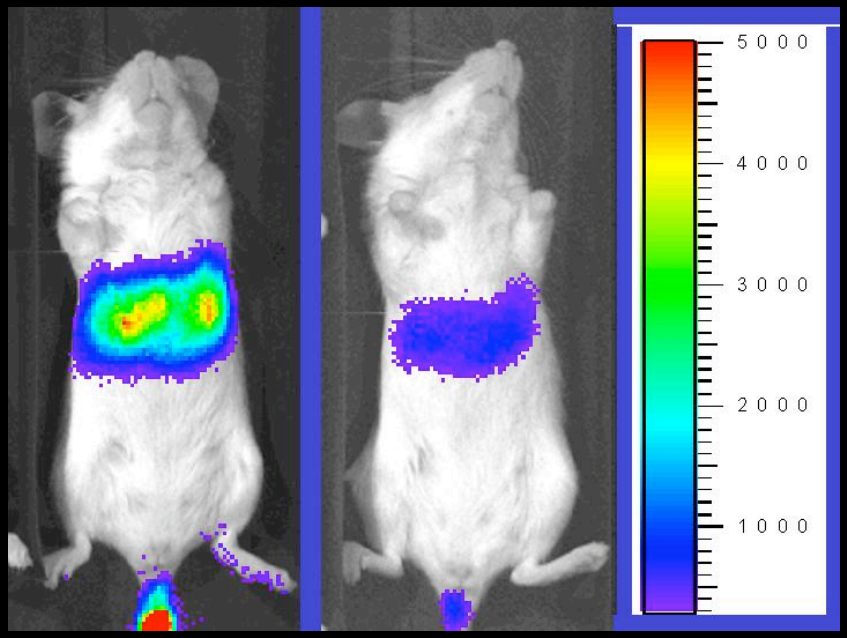
Luciferase mRNA

Luciferase Plasmid

Imaging system
Chris Contag
Contag et al.,
Photochem Photobiol
(66) 523-531 (1997)

Hydrodynamic Transfection Liu et al. and Zhang et al.

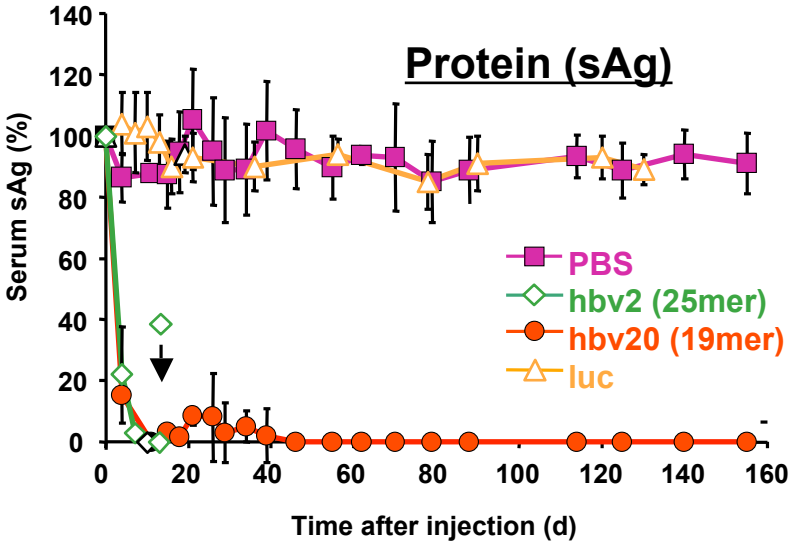
Transfection with an NS5B siRNA reduces luciferase expression from a luciferase-NS5B reporter plasmid



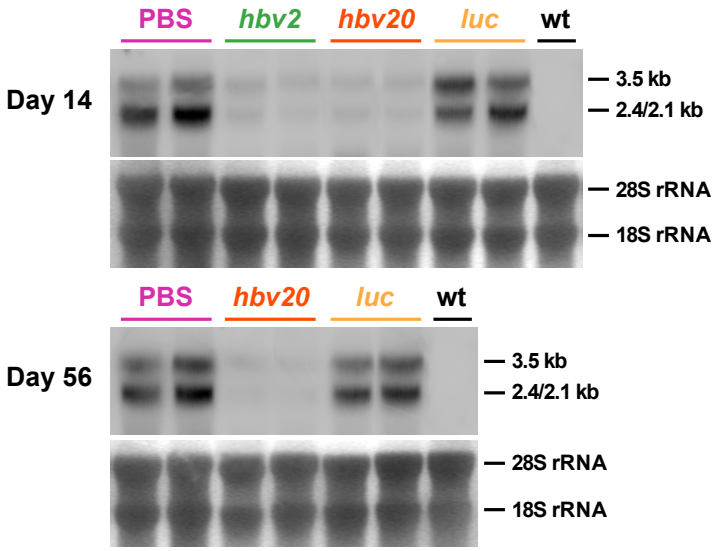
Reporter Alone

NS5B siRNA

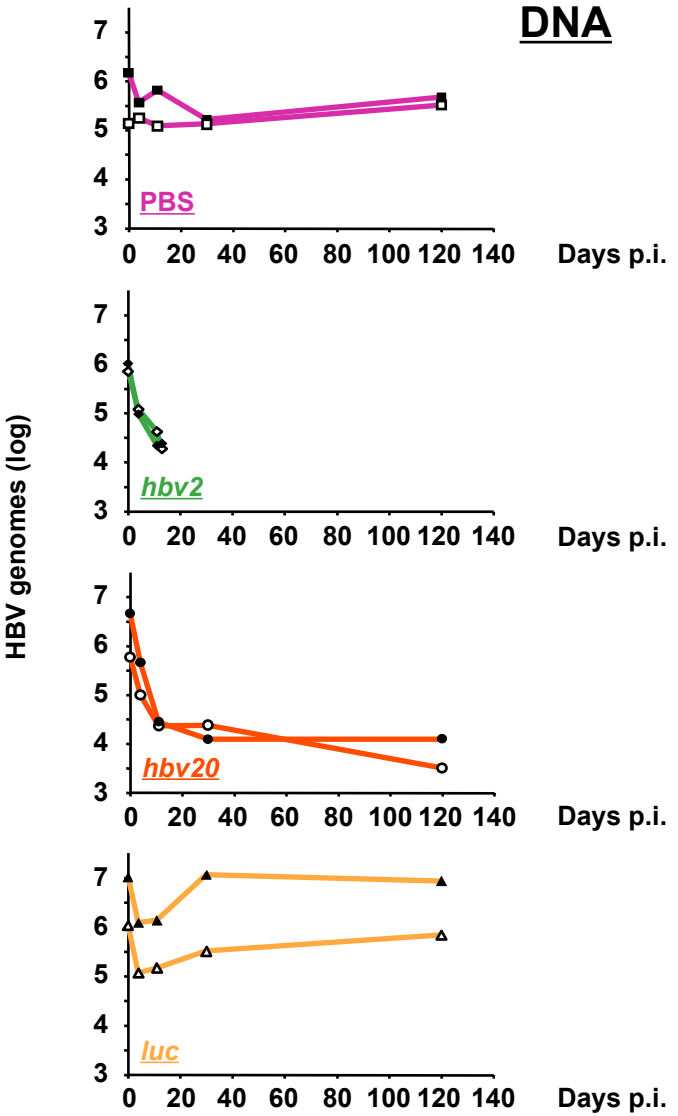
AAV-shRNA Therapy in HBV Transgenic Mice



RNA



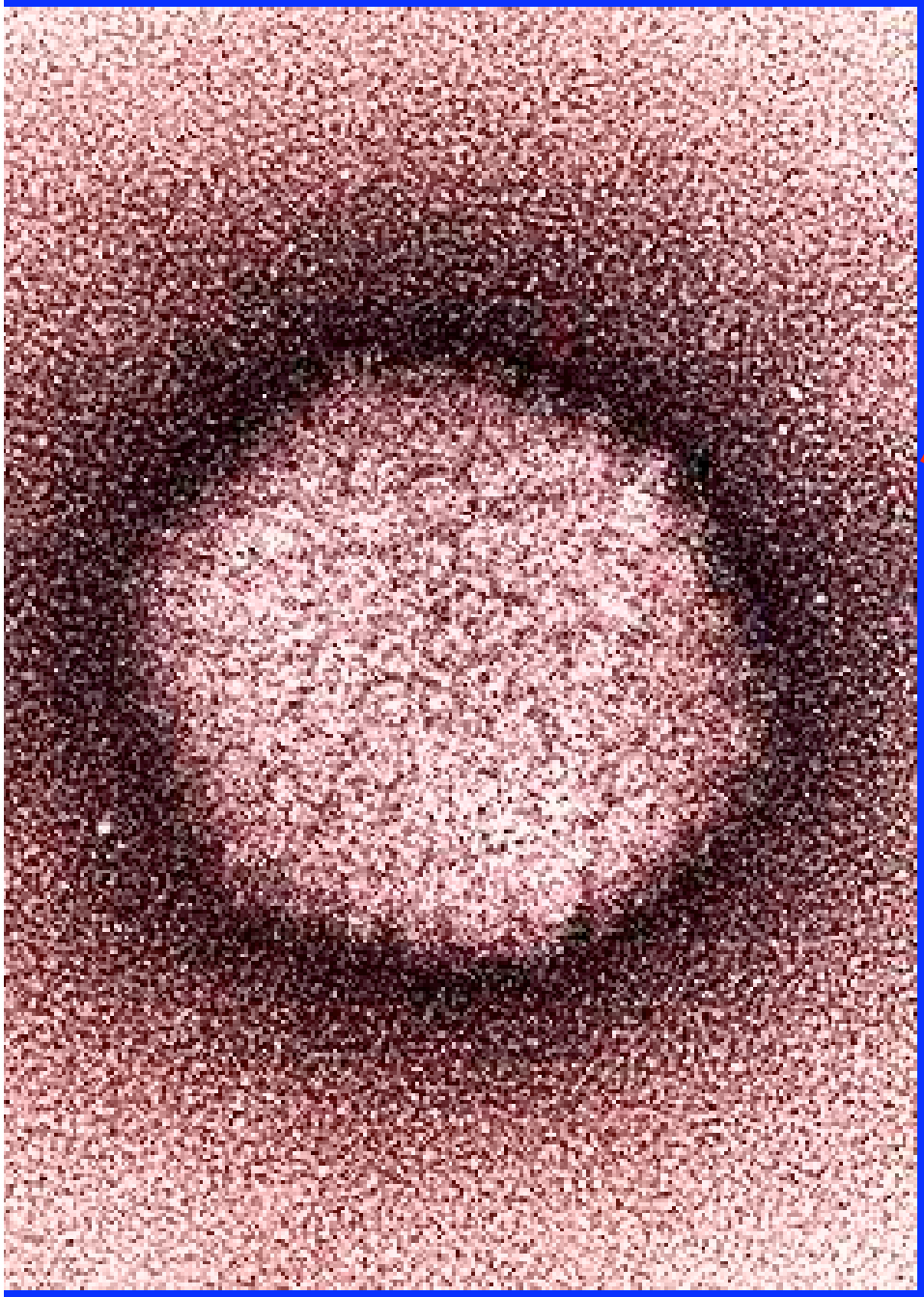
DNA



Regulation of Gene Transfer

Tissue restricted expression-regulate the gene

Alter the viral particle to enter specific cells



ligand

Cell
receptor

cell

Controlling gene expression

Regulatory elements	**Therapeutic gene**

The regulatory sequences can be engineered to be turnedon or off under specific conditions:

Administration of drugs

Within a specific cell type

By metabolic signals (e.g. glucose, low oxygen, etc)

Where do we go from here?

Gene Therapy is not a slam dunk

Still a great need for vector developmen

Studies on vector/host interactions