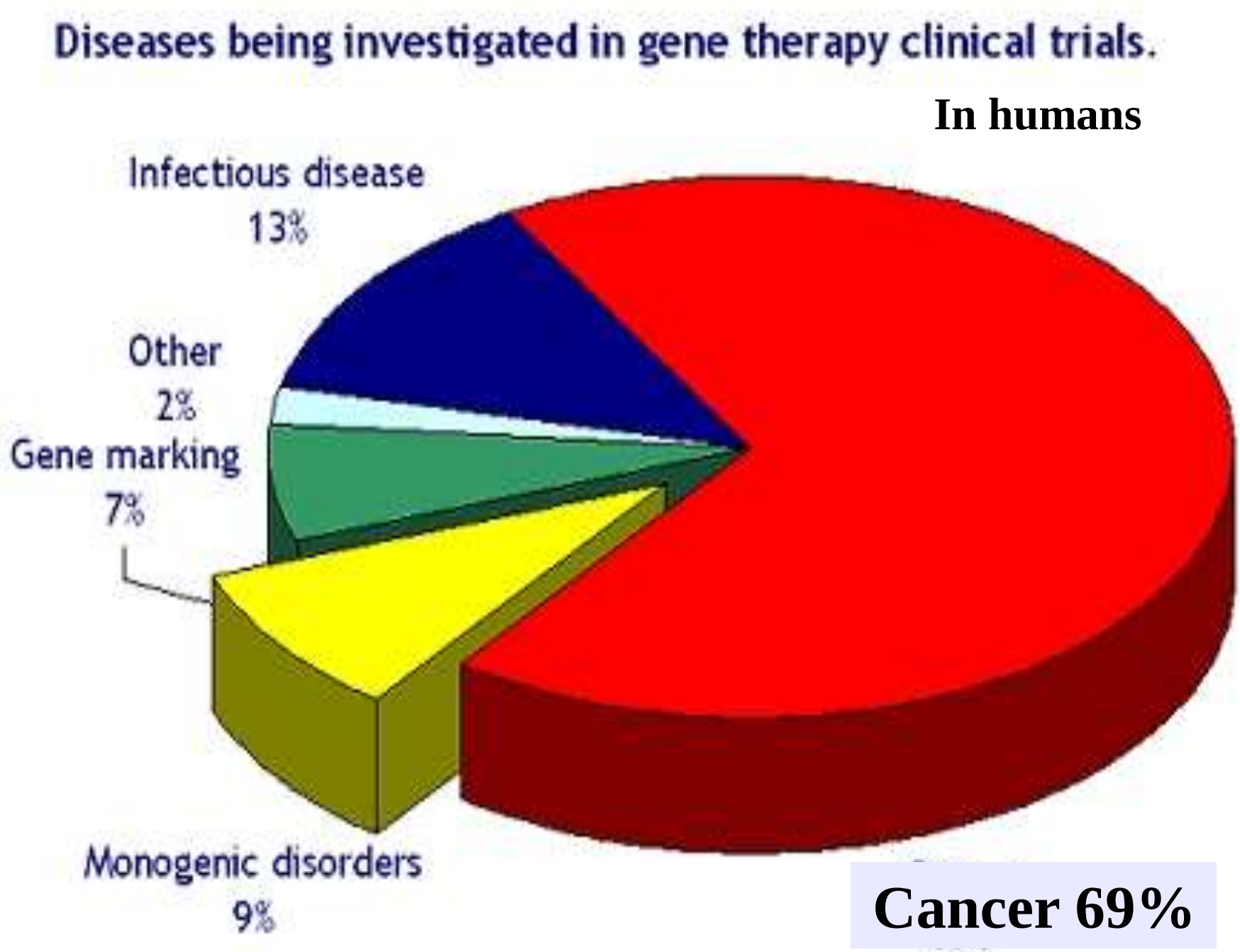
GENE THERAPY


Diseases being investigated in gene therapy clinical trials.
In humans
Infectious disease
13%
Other
2%
Gene marking
7%
Monogenic disorders
9%
Cancer 69%

General concerns

The Food and Drug Administration (FDA) has not yet approved any human gene therapy product for sale.

Four major problems with gene therapy:

1) Short-lived nature of gene therapy. Very hard to achieve any long-term benefits without integration and even with it.

2) Immune response. It reduces gene therapy effectiveness and makes repetitive rounds of gene therapy useless

3) Problems with viral vectors . Toxicity, immune and inflammatory responses, also fears that viral vector may recover disease-causing ability

4) Multigene disorders. Most commonly occurring disorders, such as heart disease, Alzheimer's disease, arthritis, and diabetes, **are caused by the combined effects of variations in many genes.**

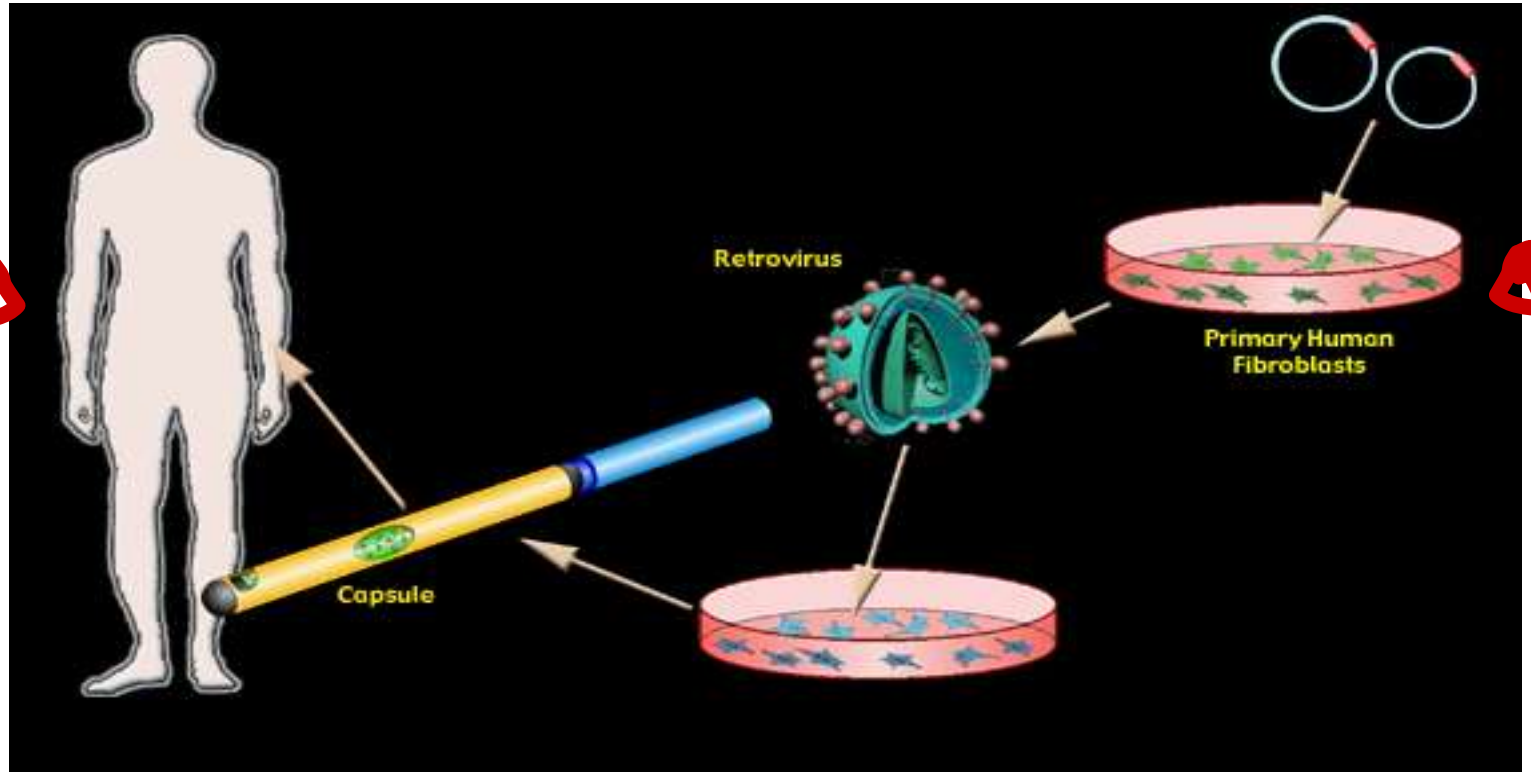
Gene therapy could be very different for different diseases

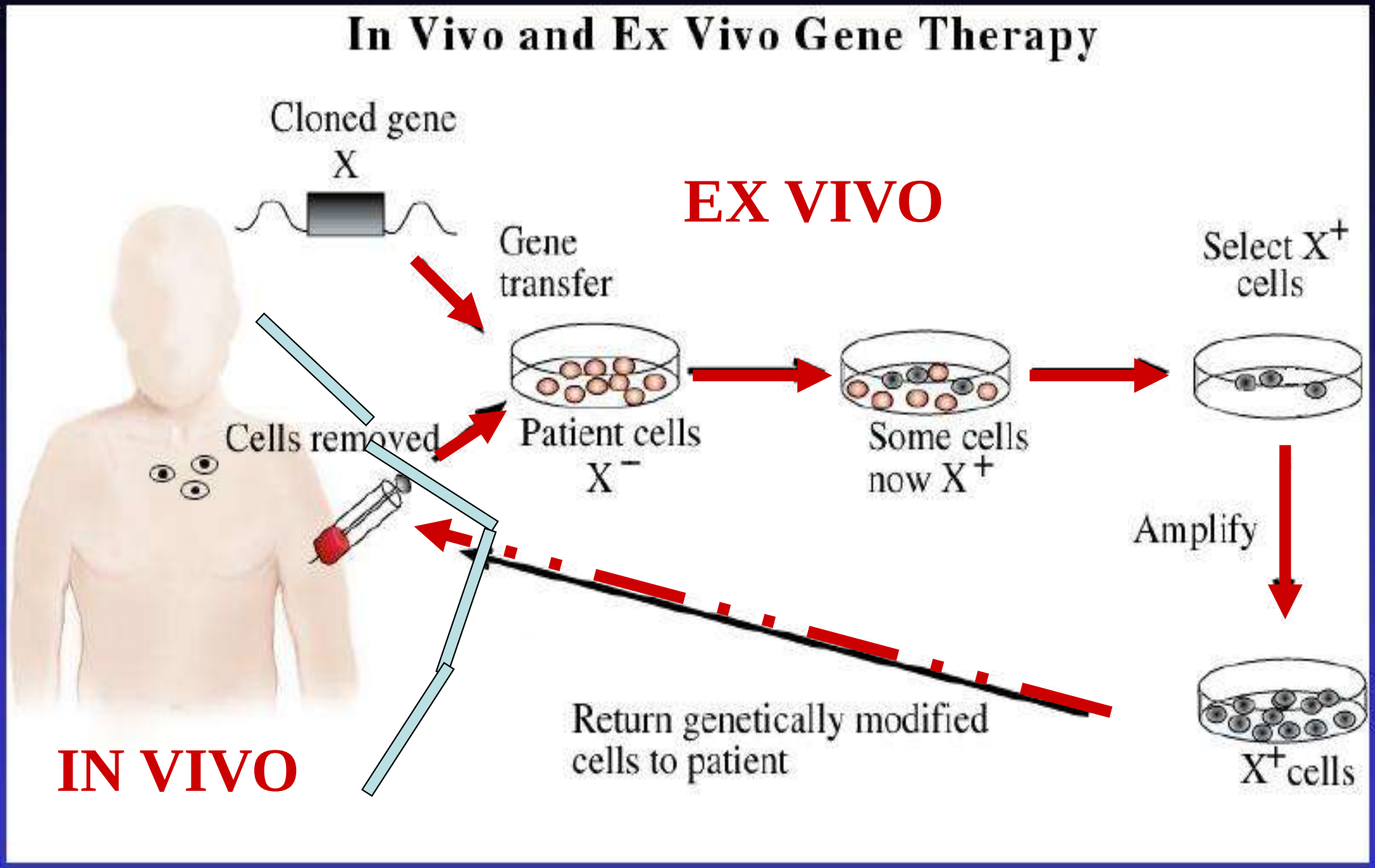
- Gene transplantation
  (to patient with gene deletion)
- Gene correction
  (To revert specific mutation in the gene of interest)
- Gene augmentation
  (to enhance expression of gene of interest)
- Targeted killing of specific cells by introducing killer gene
- Gene ablation – targeted inhibition of gene expression

Gene therapy

In vivo

Ex vivo





http://laxmi.nuc.ucla.edu:8237/M288/SChow_4_10/sld005.htm

Transgenes

Integrated

- stable expression;
may provide a cure

- random insertions
in heterochomatin
can be inactivated;

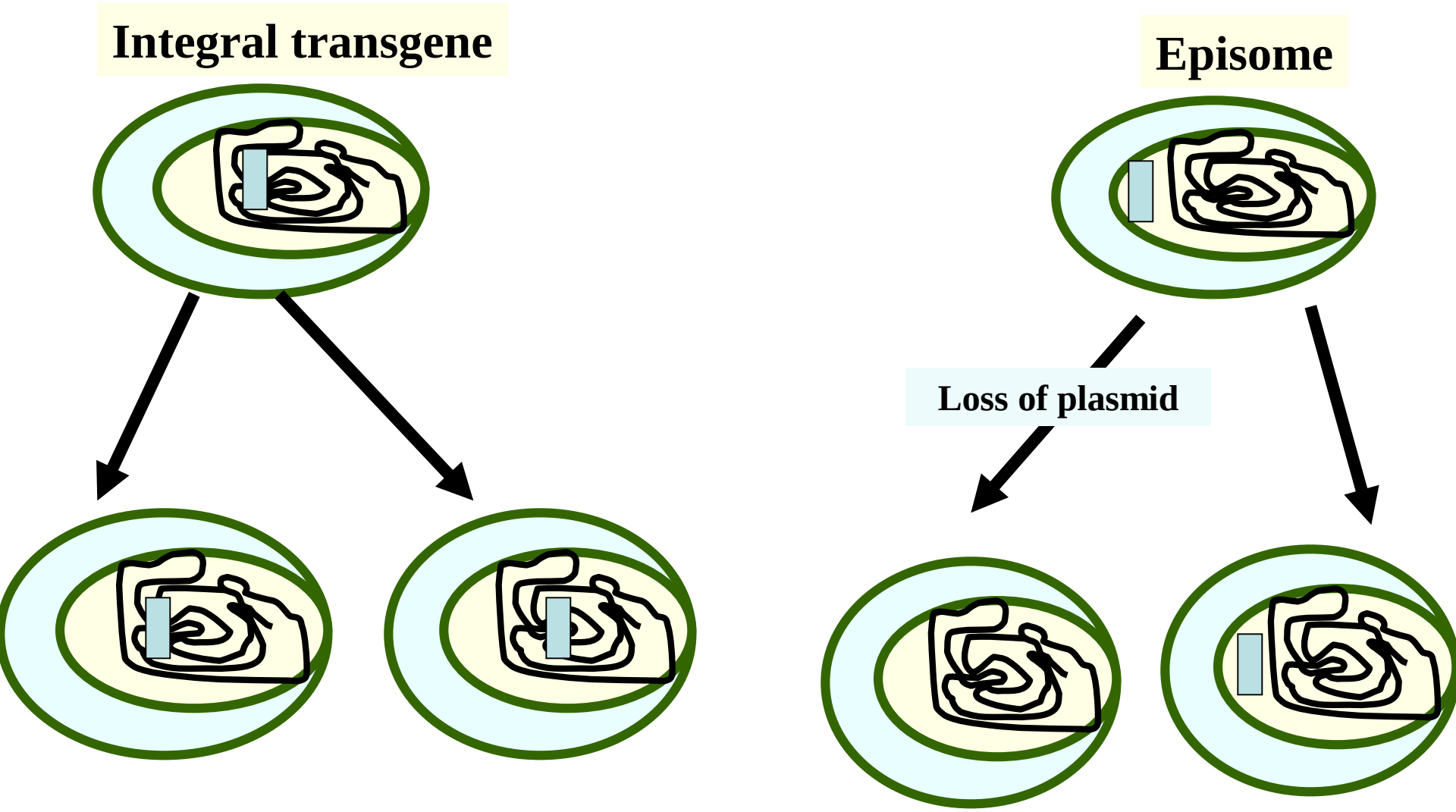
In euchromatin --
Can disrupt important host genes;
Long-term consequences are unknown

Not integrated

for episomes (plasmids)
random mutagenesis
not an issue

- expression is transient;
repeated treatments
necessary

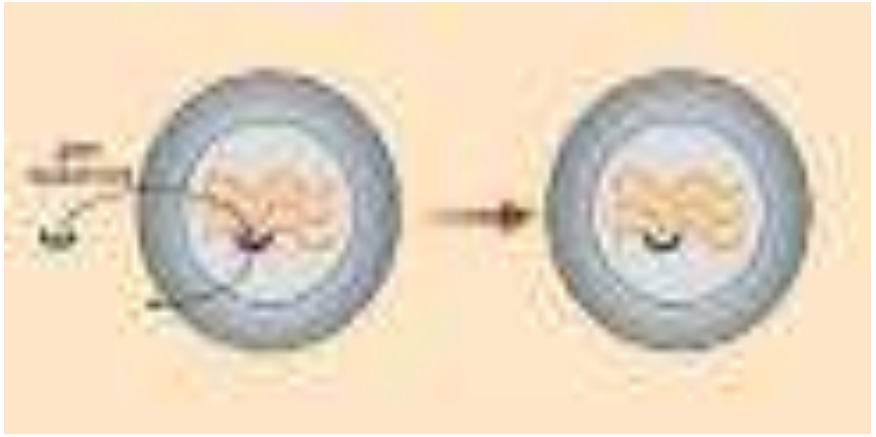
How episomes and integrated trasgenes behave in dividing cells



Influences on choice of vector

high efficiency viral vectors → **for gene replacement**

therapy of monogenic diseases (cystic fibrosis; SCID; hemophilia…)



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short term gene expression → **To prime an immune response**

…Liposomal Delivery… → **To sensitize cells to radiotherapy**

Desirable characteristics of gene delivery vector

1. **High titer or concentrations (>10^8 particles/ml)**
2. **Easy and reproducible method of production**
3. **Precise and stable introduction of transgene**
4. **Vector should not elicit immune response in the host**
5. **Transgene should be responsible for its regulatory elements (on/off system)**
6. **Vector should be able to target specific cell types**

Methods of gene delivery (therapeutic constructs)

-- **Injection of naked DNA** into tumor by simple needle and syringe

-- DNA coated on the **surface of gold pellets** which are air-propelled into the epidermis **(gene-gun),** mainly non applicable to cancer

-- DNA transfer by liposomes
(delivered by the intravascular, intratracheal, intraperitoneal or intracolonic routes)

-- **Biological vehicles (vectors)** such as viruses and bacteria.
Viruses are genetically engineered
so as not to replicate once inside the host.
They are currently the most efficient means of gene transfer.

MOST COMMON VIRAL VECTORS

Retroviruses

can create double-stranded DNA copies of their RNA genomes. Can integrate into genome. HIV, MoMuLV, v-src, Rous sarcoma virus

Adenoviruses

dsDNA viruses that cause respiratory, intestinal, and eye infections in humans. Virus for common cold

Adeno-associated viruses

ssDNA viruses that can insert their genetic material at a specific site on chromosome 19

Herpes simplex viruses

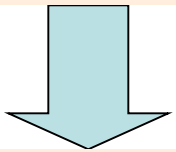
dsDNA viruses that infect a neurons. Cold sores virus

Retroviral vectors are able to infect dividing cells only

Preintegration complex of retroviruses non able to penetrate nuclear membrane.

In dividing cells nuclear membranes are broken down, so viral genome can enter and integrate into the chromosome

Infection of dividing cells only



Good for cancer gene therapy

Nevertheless, retroviruses are most often used vectors for common disease gene therapy

Every therapeutic construct should include safety features

Drawbacks of using a pseudotyped retroviral vectors

1. **Host range now is too broad.**

Cell-specific targeting not possible,
but we can use it for ex vivo approaches.

2. G protein of VSV is very immunogenic
(so, it's one-time approach)

3. G protein of VSV is toxic for cell
pseudotypes could be produced only by already dying packaging cells
(overcome by inducible promotors)

Other pseudotypes are available:

HFV – human foamy virus, HIV-1,
LCMV (lymphocytic chiriomeningitis) – non toxic for cells

Lentiviral vectors

Lentiviruses are retroviruses that can infect both dividing and nondividing cells

Preintegration complex of lentiviruses can get through the intact membrane of the nucleus of the target cell.

Able to infect nondividing or terminally differentiated cells such as neurons, macrophages, hematopoietic stem cells, retinal photoreceptors, and muscle and liver cells

Example of lentiviruses:
HIV-1 (infects T-helper cells) – AIDS.

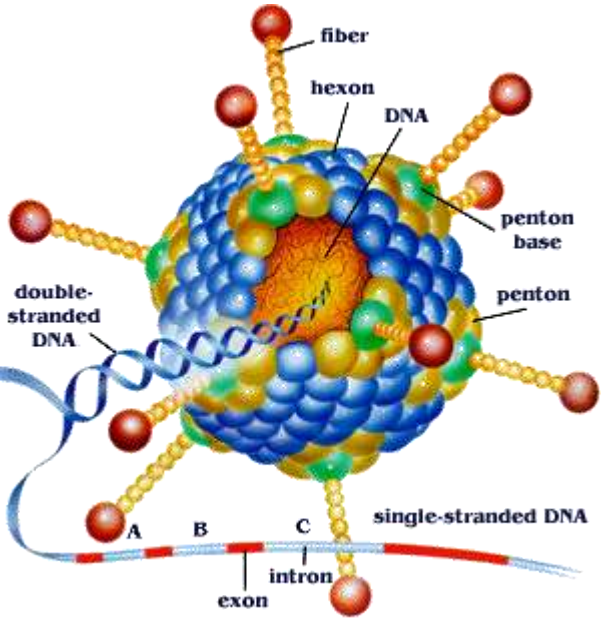
Good feature – no immune response!

ADENOVIRUSES

non-enveloped viruses
containing a linear double stranded DNA genome

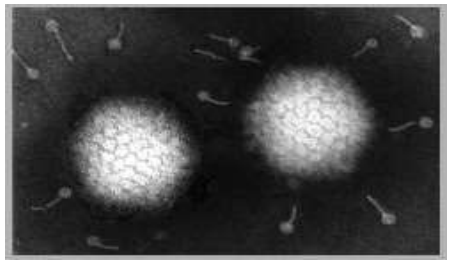
40 serotypes known;
most producing respiratory infections in humans

subgroup C serotypes 2 or 5 are predominantly used as vectors



www.nobel.se

can infect both
dividing and nondividing cells

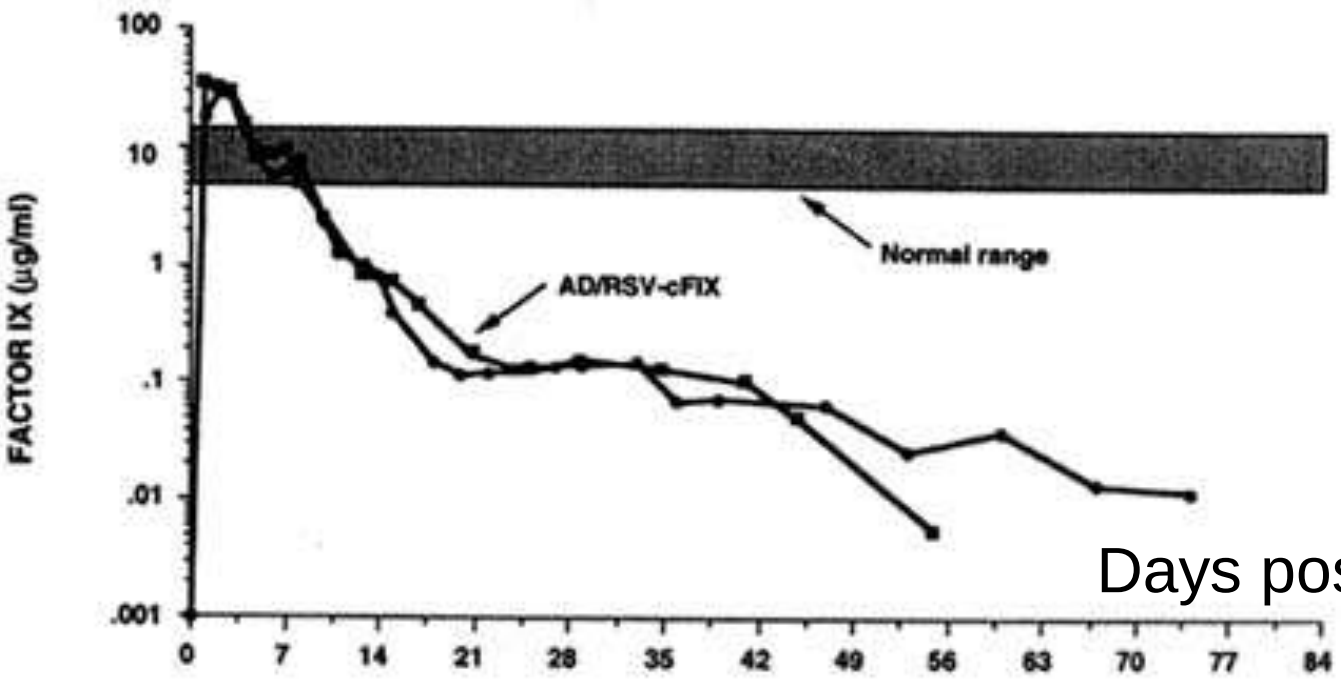


12 antenna-like fiber projections
for virus attachment

Problems with adenoviral vectors

1. Cannot integrate with the host cell genome

expression from adenoviral vectors is transient (5-10 days) **due to immunoclearance of the virus**



in vivo hepatic gene delivery to hemophilia B dogs.

Adeno-associated virus (AAV)

Can be ideal as:

-- **does not stimulate inflammation** in the host

-- **does not elicit antibodies** against itself

-- **can enter non-dividing cells**

-- integrates successfully into **one spot in the genome of its host** (on chromosome 19 in humans).

How to make expression tissue specific?