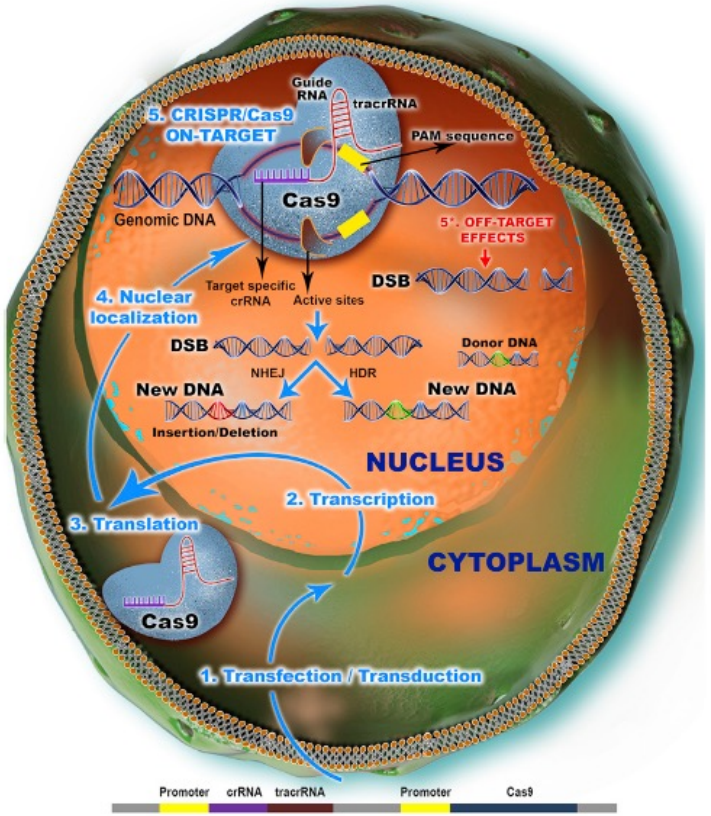
GENE THERAPY

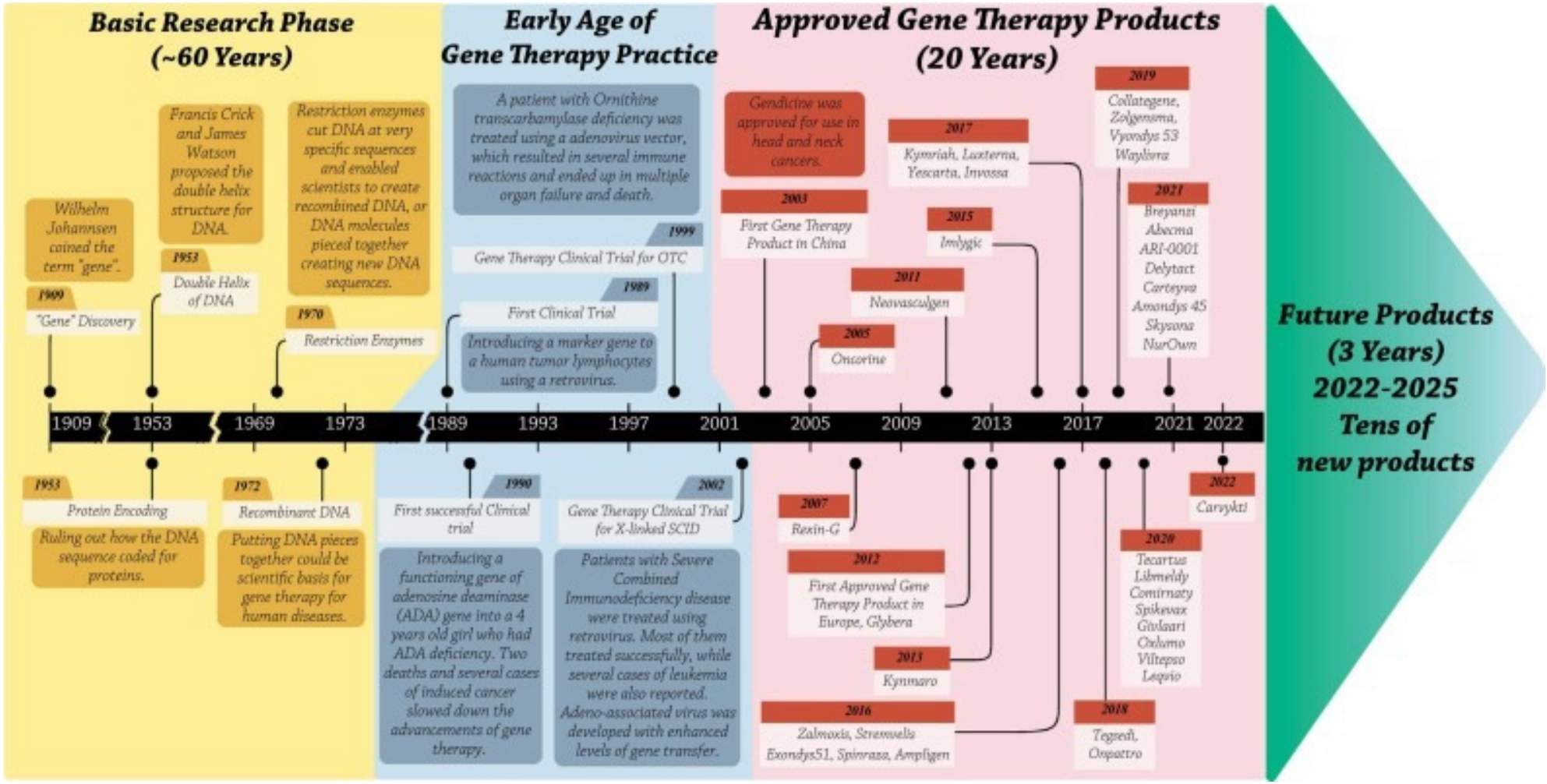


Gene therapy

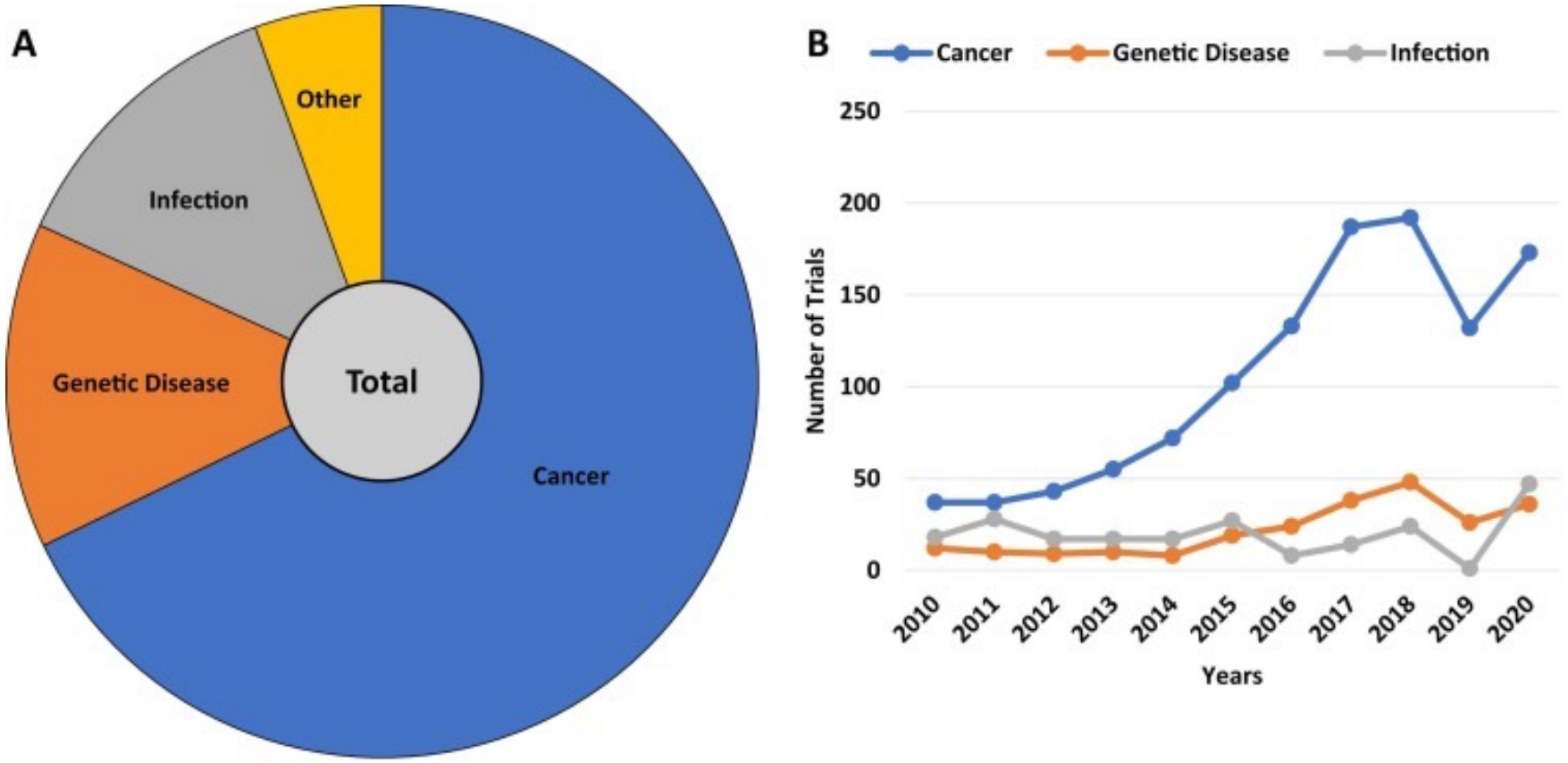
Transfer of therapeutic nucleic acid (NA) into a tissue

→ **Choice and preparation** of therapeutic NA and of its vehicle

→ **Targeting** to the desired tissue


Basic Research Phase
(~60 Years)
Early Age of
Gene Therapy Practice
Approved Gene Therapy Products
(20 Years)
Future Products
(3 Years)
2022-2025
Tens of
new products
Wilhelm Johannsen coined the term "gene".
1909
"Gene" Discovery
Francis Crick and James Watson proposed the double helix structure for DNA.
1953
Double Helix of DNA
Restriction enzymes cut DNA at very specific sequences and enabled scientists to create recombined DNA, or DNA molecules pieced together creating new DNA sequences.
1970
Restriction Enzymes
A patient with Ornithine transcarbamylase deficiency was treated using a adenovirus vector, which resulted in several immune reactions and ended up in multiple organ failure and death.
1999
Gene Therapy Clinical Trial for OTC
1989
First Clinical Trial
Introducing a marker gene to a human tumor lymphocytes using a retrovirus.
Gendicine was approved for use in head and neck cancers.
2003
First Gene Therapy Product in China
2017
Kymriah, Luxterna, Yescarta, Invossa
2019
Collategene, Zolgensma, Vyondys 53 Waylivra
2015
Imlygic
2021
Breyanzi Abecma ARI-0001 Delytact Carteyva Amondys 45 Skysona NurOwn
2011
Neovasculgen
2005
Oncorine
1909
1953
1969
1973
1989
1993
1997
2001
2005
2009
2013
2017
2021
2022
1953
Protein Encoding
Ruling out how the DNA sequence coded for proteins.
1972
Recombinant DNA
Putting DNA pieces together could be scientific basis for gene therapy for human diseases.
1990
First successful Clinical trial
Introducing a functioning gene of adenosine deaminase (ADA) gene into a 4 years old girl who had ADA deficiency. Two deaths and several cases of induced cancer slowed down the advancements of gene therapy.
2002
Gene Therapy Clinical Trial for X-linked SCID
Patients with Severe Combined Immunodeficiency disease were treated using retrovirus. Most of them treated successfully, while several cases of leukemia were also reported. Adeno-associated virus was developed with enhanced levels of gene transfer.
2007
Rexin-G
2012
First Approved Gene Therapy Product in Europe, Glybera
2013
Kynnaro
2016
Zalmoxis, Strimvelis Exondys51, Spinraza, Ampligen
2018
Tegsedi, Onpattro
2020
Tecartus Libmeldy Comirnaty Spikevax Givlaari Oxlumo Viltepso Leqvio
2022
Carvykti

Gene therapy trials and diseases

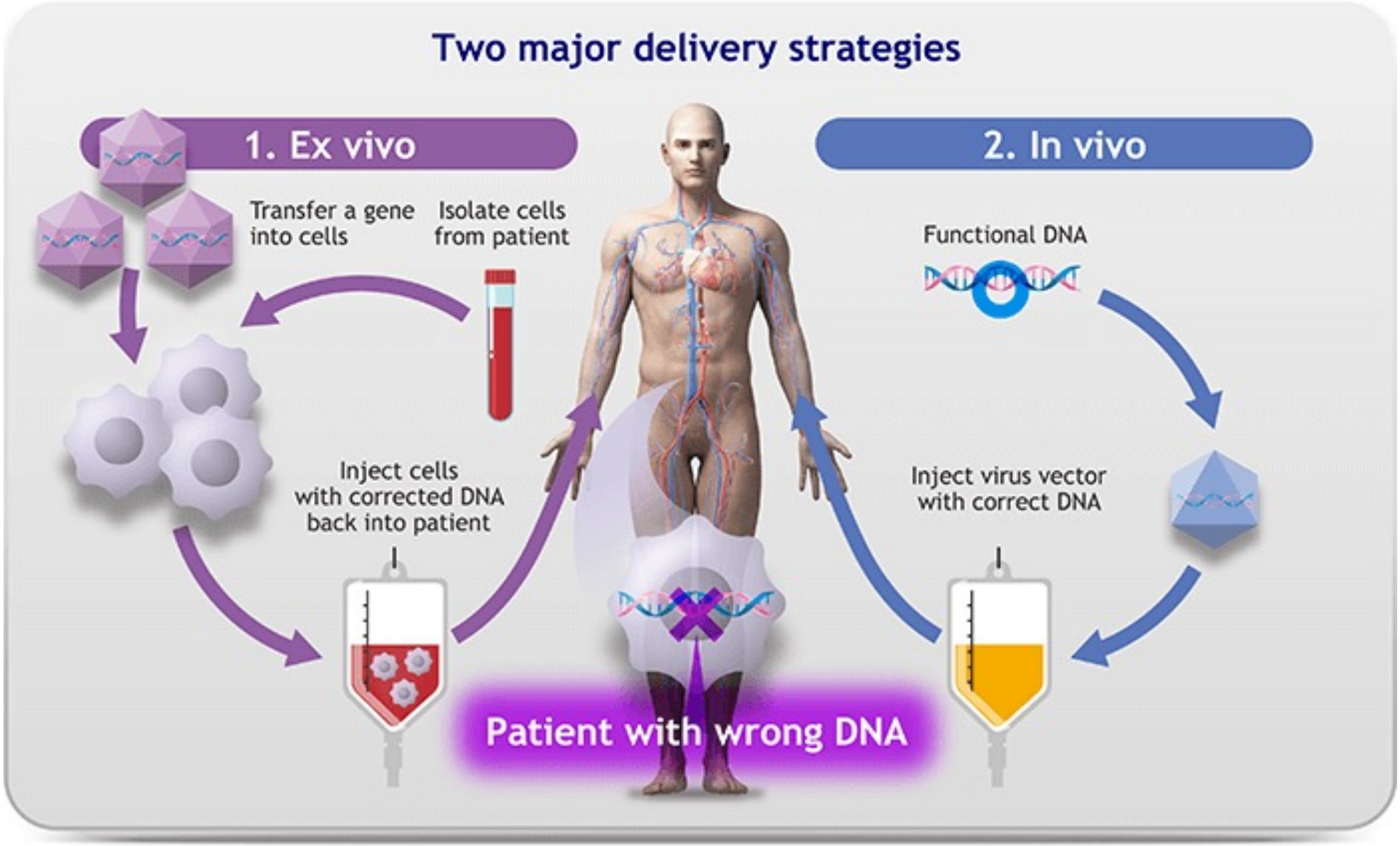


More than 5,000 patients have been treated in last ~12 years worldwide

General issues to address when planning a GT approach

- ✓What tissue should be targeted?
- ✓What type of vehicle should be used?
- ✓What's the expected duration of the effect?
- ✓What route of administration?
- ✓How safe is the approach?

Types of GT


Two major delivery strategies
1. Ex vivo
Transfer a gene into cells
Isolate cells from patient
Inject cells with corrected DNA back into patient
2. In vivo
Functional DNA
Inject virus vector with correct DNA
Patient with wrong DNA

Transgenes

Integrated

- stable expression;
may provide a cure

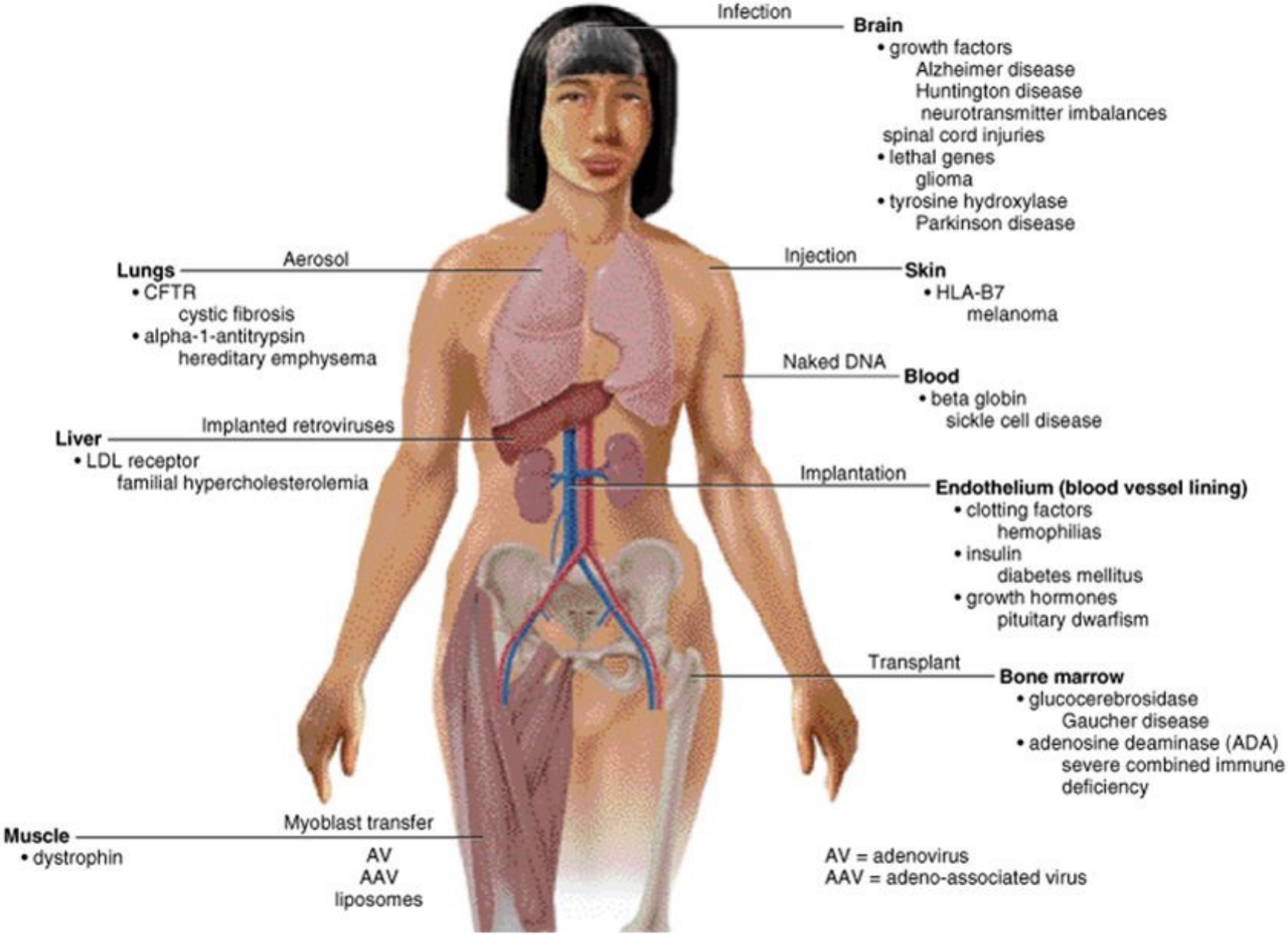
- random insertions
in heterochromatin
can be inactivated;

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

Not integrated

- expression is transient;
repeated treatments
are necessary

Target Sites for Gene Therapy



Methods of Nucleic Acid (NA) delivery

1-- Injection of naked NA into tissue by simple needle and syringe

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

3-- Viruses
Viruses are genetically engineered
so as not to replicate once inside the host.
They are currently the most efficient means of gene transfer.

Protocols by vectors

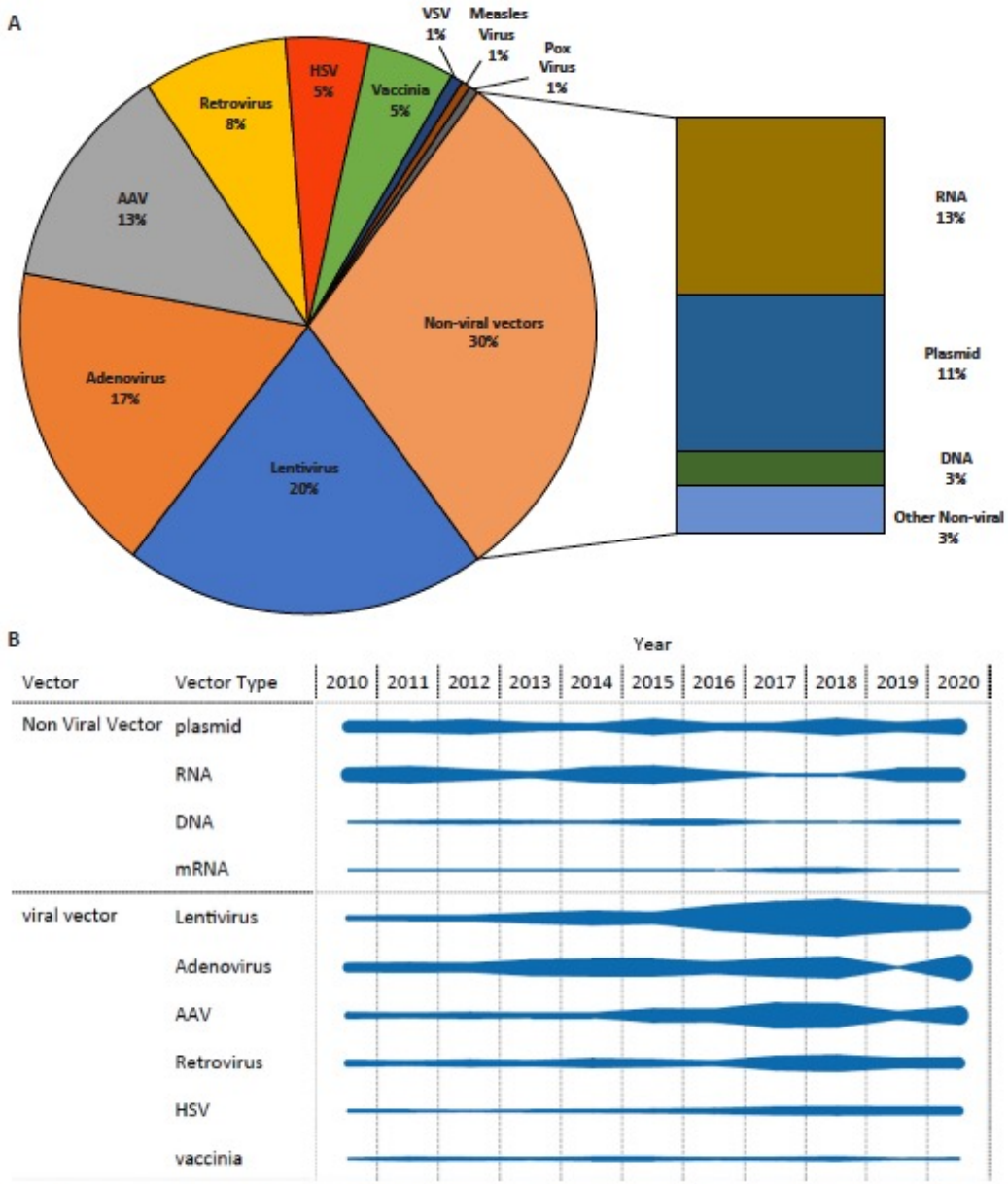
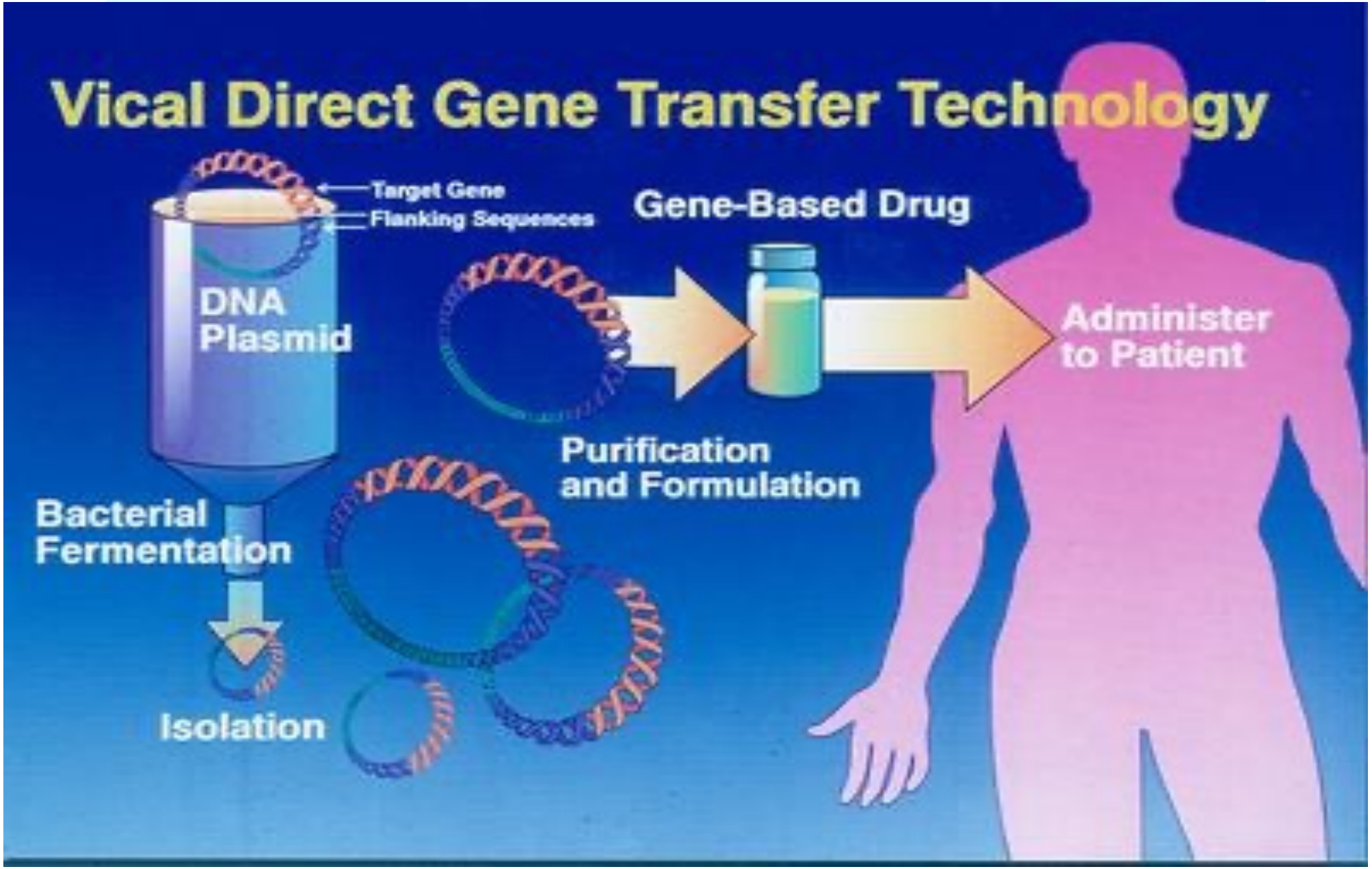
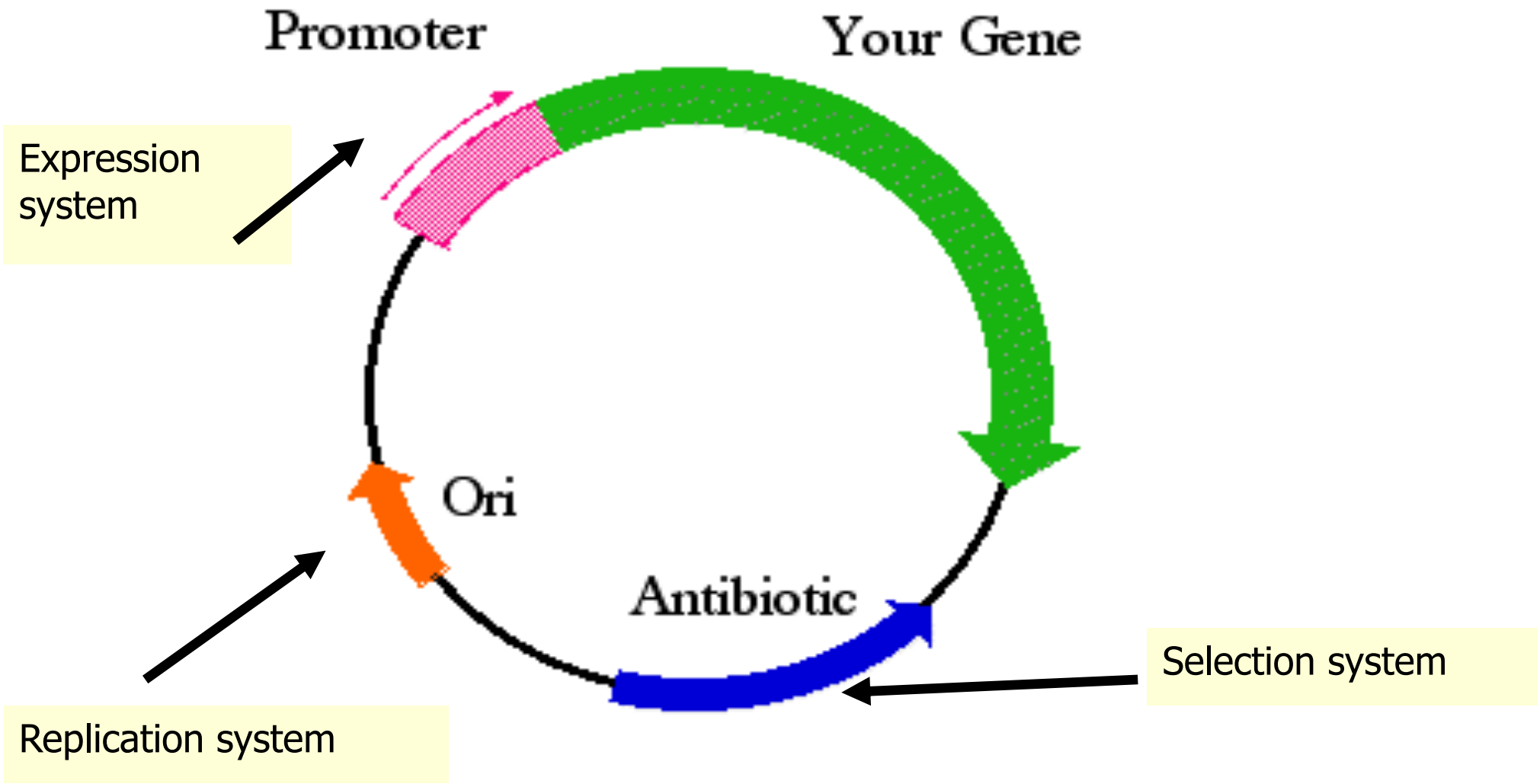


Fig. 5. Trends of using different vectors in gene therapy trials. (A) The contribution part of viral and non-viral vectors and their types in clinical trials from 2010 to 2020. Viral vectors played the main part in transferring the genetic materials, however, the characteristics of each vector made them ideal candidates for specific usages. (e.g., lentivirus & retrovirus for *ex-vivo* gene therapy, AAV for *in-vivo* gene therapy, or RNAs for loss-of-function changes.) RNAs and plasmids were the most common non-viral vectors. (B) The time trend of gene therapy clinical trials using different vectors.

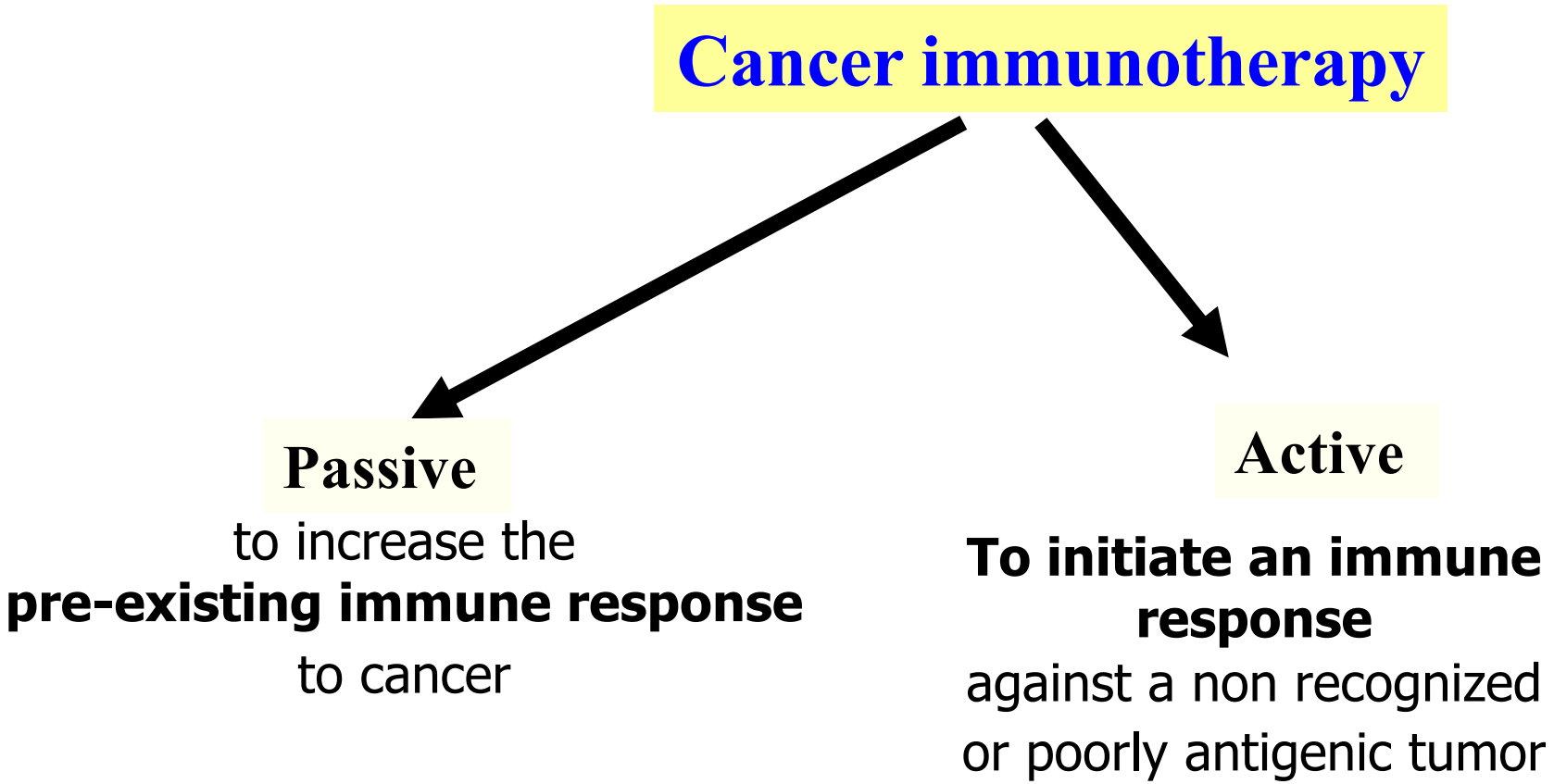
1--Injections of naked NA



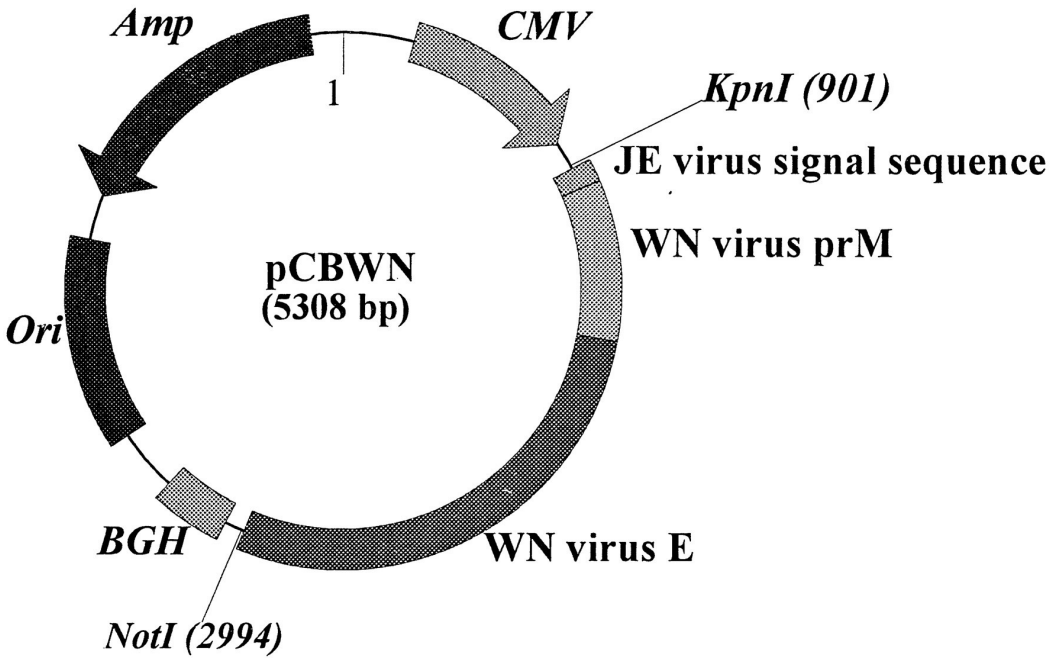
Naked DNA Vector



DNA vaccines



West Nile Virus Recombinant DNA Vaccine



There are currently **no DNA vaccines approved for human therapy.**

Only for **veterinary use** (**Nile fever in horses**)

Early 2000

Phase 1 trial for safety

GT in DMD with naked DNA

French Muscular Dystrophy Association (AFM): DMD

Phase I trial on gene transfer for Duchenne/Becker's Muscular Dystrophy

Nine patients in three groups, injected into radialis muscle:

a single injection of 200 ug of plasmid with dystrophin

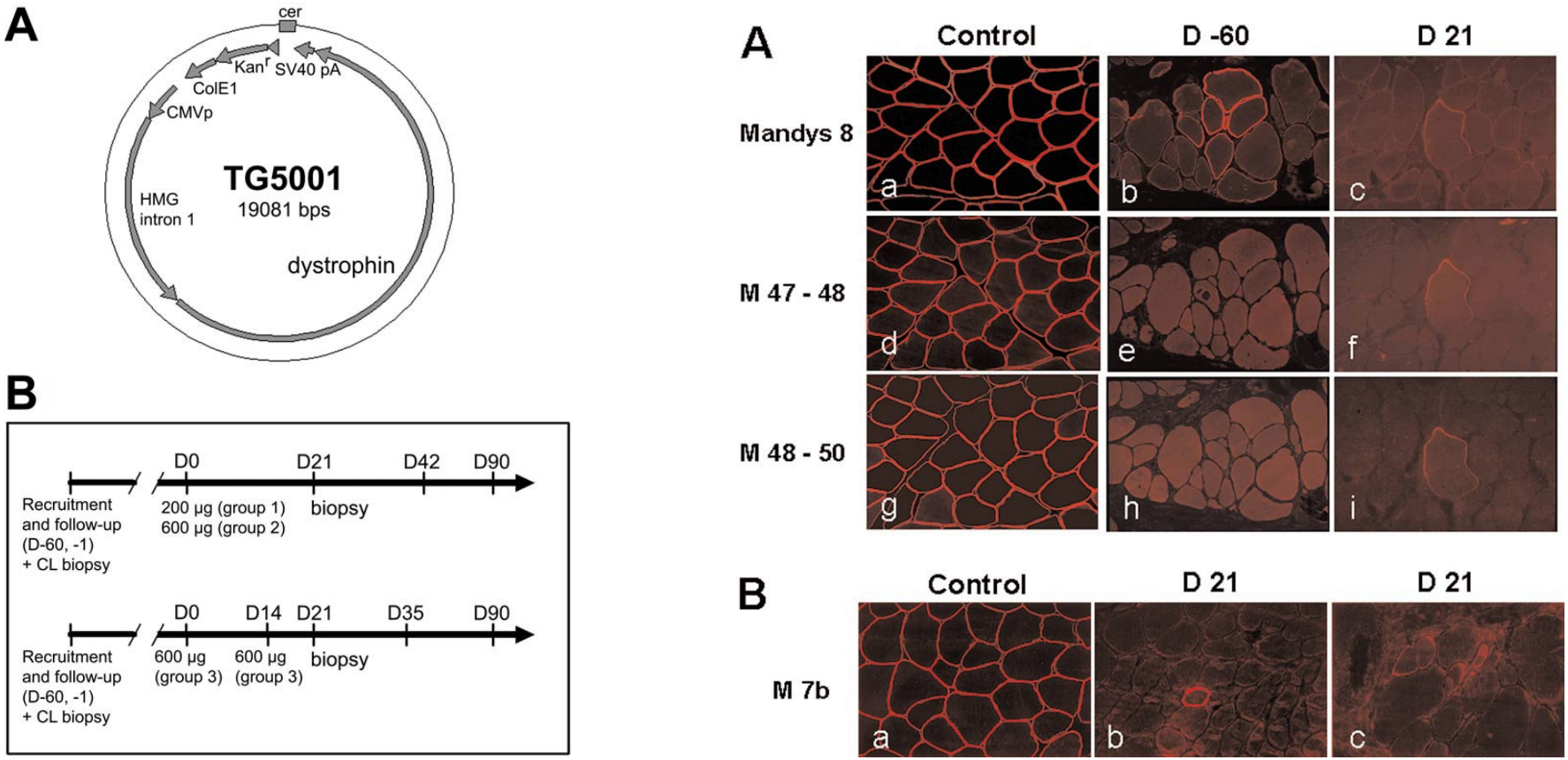
a single injection of 600 ug of plasmid with dystrophin

Two injections of 600 ug each of plasmid with dystrophin

} **Muscle segment were taken out for examination**

GT in human DMD

HUMAN GENE THERAPY 15:1065–1076 (November 2004)



Expression of dystrophin was found in 1 to 10 percent of muscle fibers for 50% of groups 1 and 2 **; for all 3 patients in group 3**

No immune reactions; no side effects

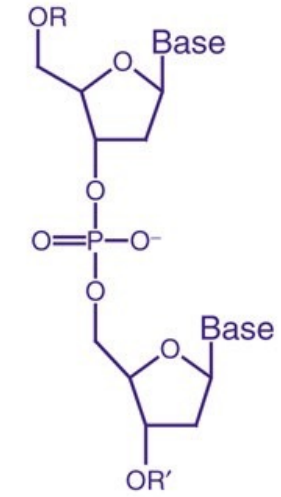
Abstract

Nine patients with Duchenne or Becker muscular dystrophy were injected via the radialis muscle with a full-length human dystrophin plasmid, either once with 200 or 600 microg of DNA or twice, 2 weeks apart, with 600 microg of DNA. In the biopsies taken 3 weeks after the initial injection, the vector was detected at the injection site in all patients. Immunohistochemistry and nested reverse transcription-polymerase chain reaction indicated dystrophin expression in six of nine patients. The level of expression was low (up to 6% weak, but complete sarcolemmal dystrophin staining, and up to 26% partial sarcolemmal labeling). No side effects were observed, nor any cellular or humoral anti-dystrophin responses.

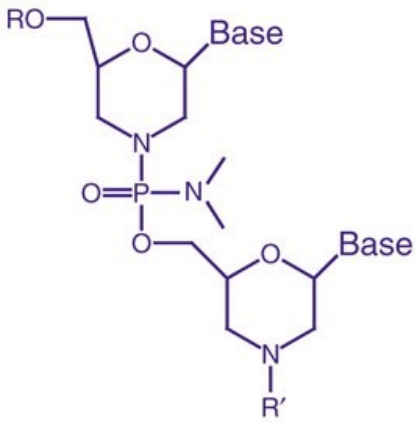
These results suggest that exogenous dystrophin expression can be obtained in Duchenne/Becker patients after intramuscular transfer of plasmid, without adverse effects, hence paving the way for future developments in gene therapy of hereditary muscular diseases.

HUMAN GENE THERAPY *15:1065–1076 (November 2004)*

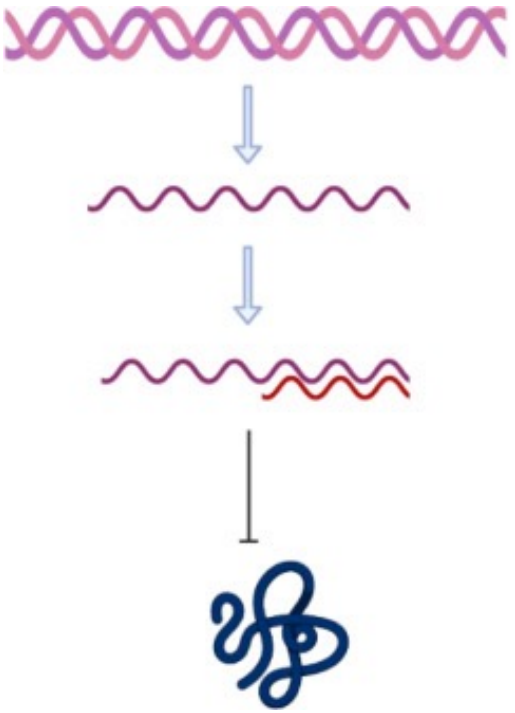
GT with Morpholino Oligonucleotides



Phosphodiester DNA



Morpholino



In vivo GT of DMD: Eteplirsen

- **Exondys 51** (Sarepta Therapeutics: approved in 2016 by the FDA
- 30 nt **phosphorodiamidate morpholino oligomer (PMO)** that promotes the cutting and exclusion of mutated exon 51 (premature stop) during the splicing process of the dystrophin pre-mRNA. By binding to the exon, it blocks the binding of a protein that regulates splicing
- In this way (**exon skipping**) the exon is lost but the reading frame is restored.
- Administered by **venous infusion (*in vivo*)**
- Indicated for patients with exon 51 mutation, which affects about **13% of patients with DMD**.
- Two other drugs act in a similar way:
  - **Golodirsen**: FDA approved in 2019, exon 51
  - **Viltolarsen**: FDA approved in 2020, exon 53

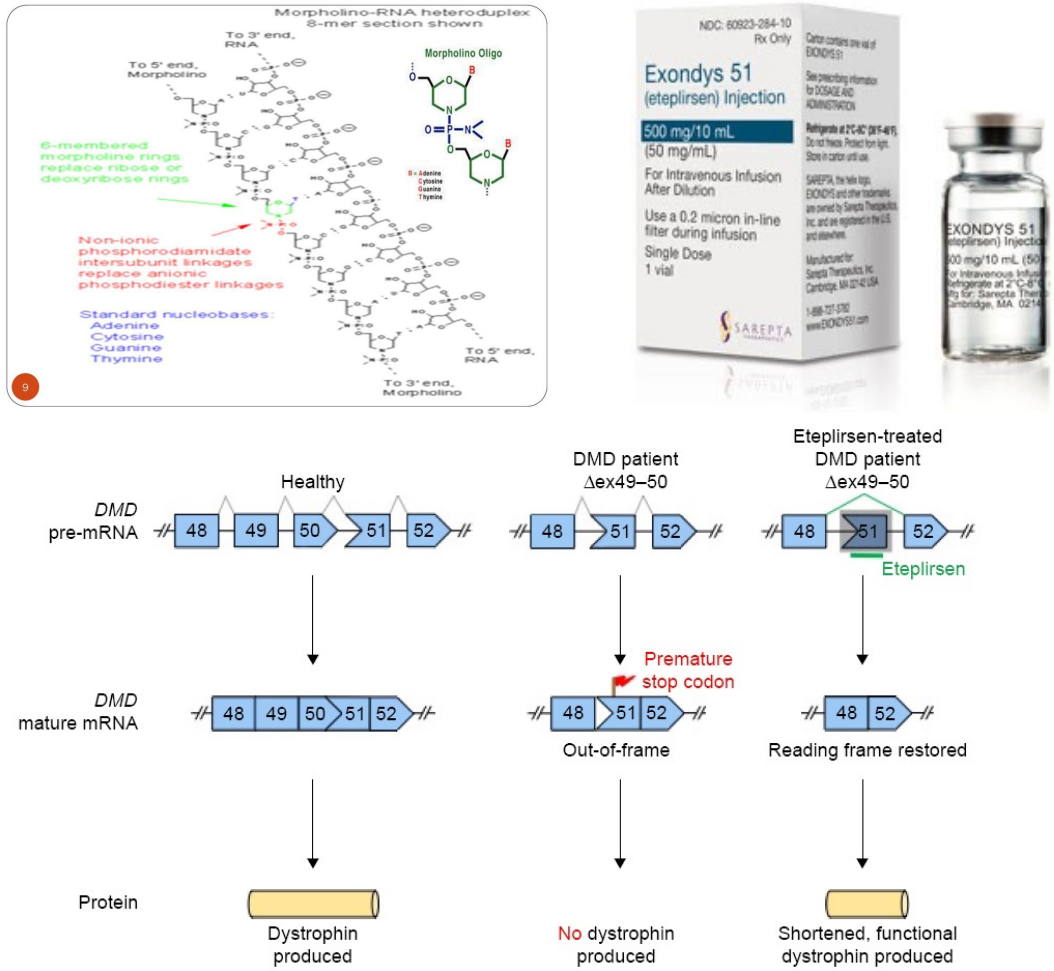
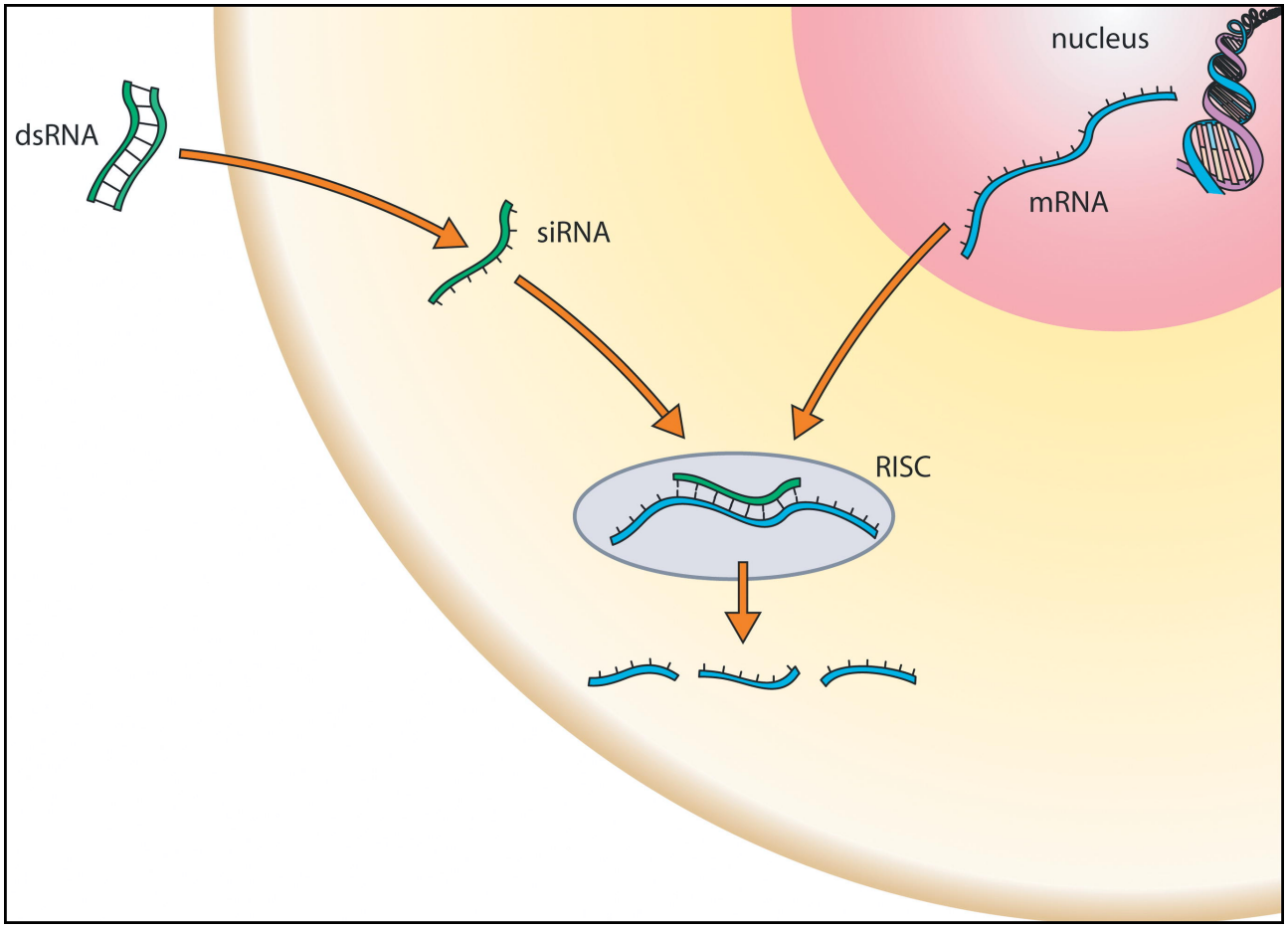


Figure 1 Eteplirsen is an exon-skipping therapeutic.
Notes: Eteplirsen (green bar) specifically recognizes exon 51 of the *DMD* gene. Upon binding, it influences the splicing machinery to skip exon 51 from the mature mRNA transcript. This restores the reading frame of *DMD*, allowing for successful translation of a shortened but functional dystrophin protein. Shown above is a case where eteplirsen is used to treat a DMD patient with a deletion spanning exons 49 and 50. This creates an out-of-frame frameshift that introduces a premature stop codon and results in nonproduction of dystrophin.
Abbreviations: DMD, Duchenne muscular dystrophy; mRNA, messenger RNA.

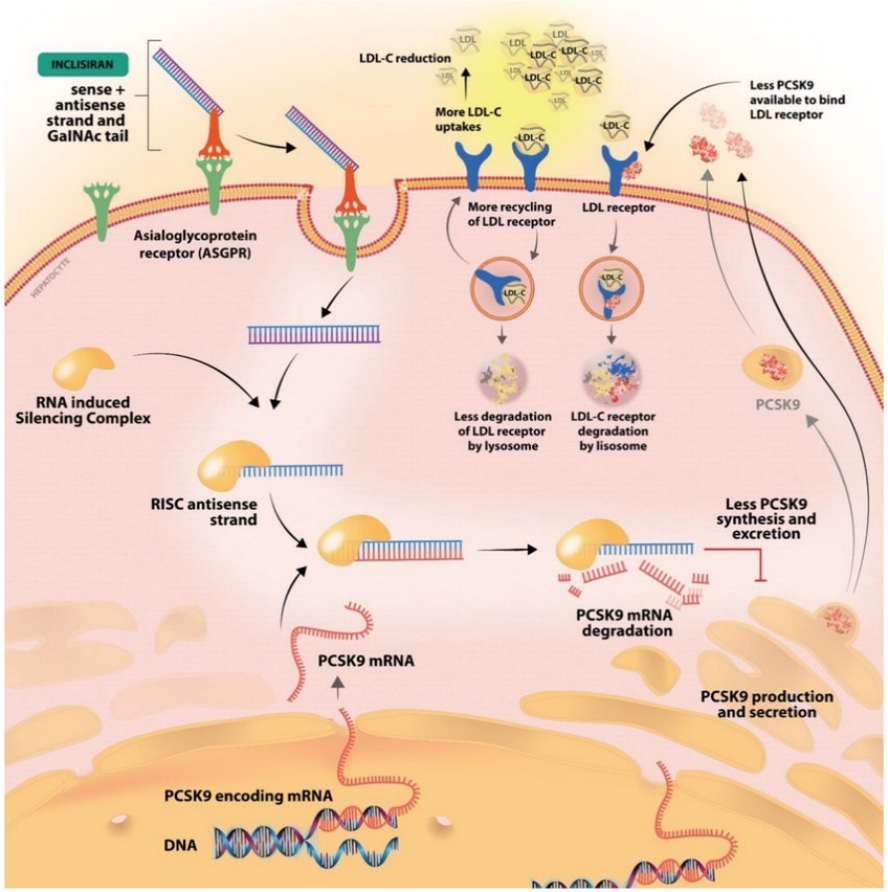
5’-CTCCAACATCAAGGAAGATGGCATTTCTAG-3’

Small Interfering RNA (siRNA)



In vivo GT of FH: Leqvio

- INCLISIRAN (Leqvio®)
- Approved by **AIFA 3-10-2022** to treat heterozygous **familiar or non familiar <u>hypercholesterolemia</u>**
- Prescribed in association to statins, or in case of statin intolerance
- PCSK9 binds LDL receptor and promotes its degradation
- DS **siRNA directed against PCSK9**, conjugated to triantennary-acetylgalactosamine **GalNAc (binds to ASGPR** (asialoglycoprotein receptor), highly expressed in hepatocytes)
- **Causes PCSK9 downregulation, thus preventing LDLR degradation**
- **Injected subcutaneously twice a year** (in vivo)



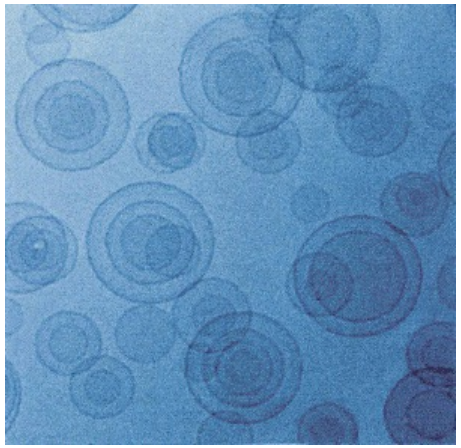
siRNAs approved and in phase 3

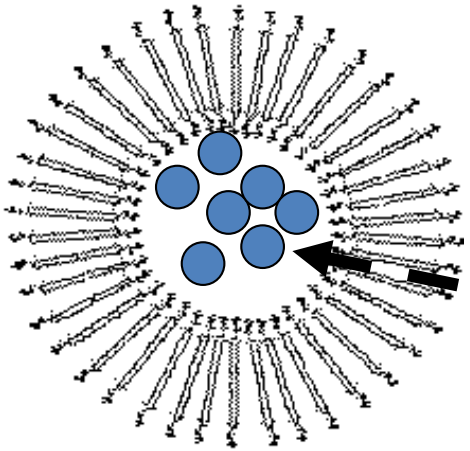
Drug Name	Delivery Platform/Targeting Ligand	Disease/Targeting Gene	Company	Updated Status
Onpattro (Patisiran)	LNP-siRNA	TTR-mediated amyloidosis (Transthyretin)	Alnylam	FDA. approval (10/10/2018)
Givlaari (Givosiran)	GalNAc-siRNA	Acute hepatic porphyria (delta-aminolevulinate synthase 1)	Alnylam	FDA approval (11/20/2019)
Oxlumo (Lumasiran)	GalNAc-siRNA	Primary hyperoxaluria type 1 (hydroxy acid oxidase 1)	Alnylam	FDA approval (11/23/2020)
Leqvio (Inclisiran)	GalNAc-siRNA	Hypercholesterolemia (proprotein convertase subtilisin/Kexin type 9)	Alnylam Novartis	FDA approval (12/22/2021)
Vutrisiran (ALN-TTRSC02)	GalNAc-siRNA	TTR-mediated amyloidosis (Transthyretin)	Alnylam	Phase III
Fitusiran (ALN-AT3SC)	GalNAc-siRNA	Haemophilia A and B and rare blood disorders (antithrombin)	Alnylam, Sanofi, Genzyme	Phase III
Nedosiran (DCR-PHXC)	GalNAc-siRNA	Primary hyperoxaluria (lactate dehydrogenase A)	Alnylam, Dicerna	Phase III
Teprasiran (QPI-1002)	None	Acute kidney injury (tumor protein)	Quark, Novartis	Phase III
Cosdosiran (QPI-1007)	None	NAION and glaucoma (Caspase 2)	Quark,	Phase III
Tivanisiran (SYL1001)	None	Ocular pain and dry eye disease (TRPV1)	Sylentis	Phase III

2022

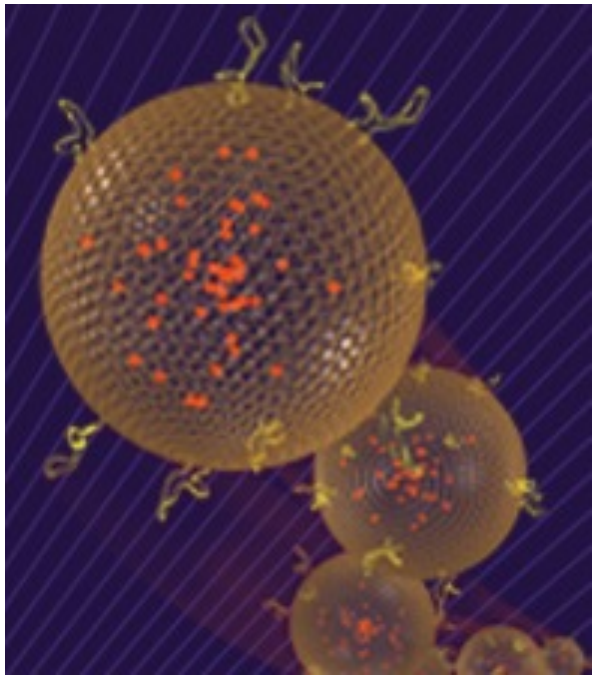
2--Liposomes

Spherical vesicle with a lipid bilayer



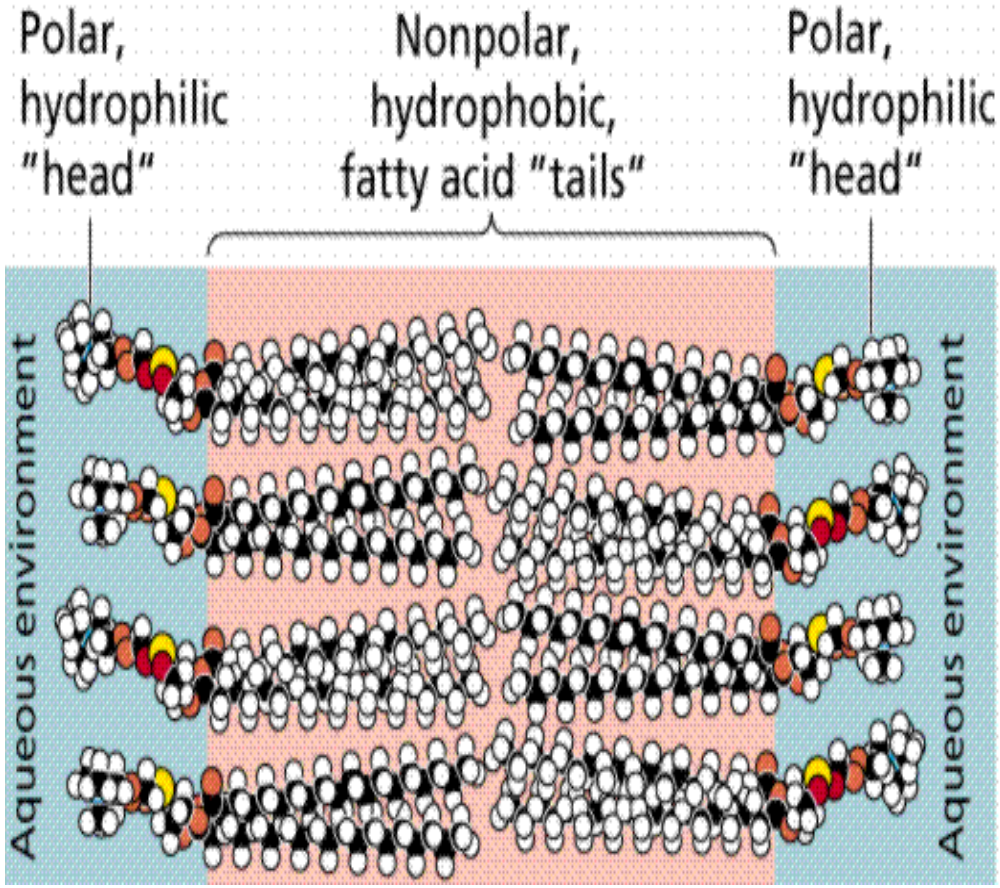


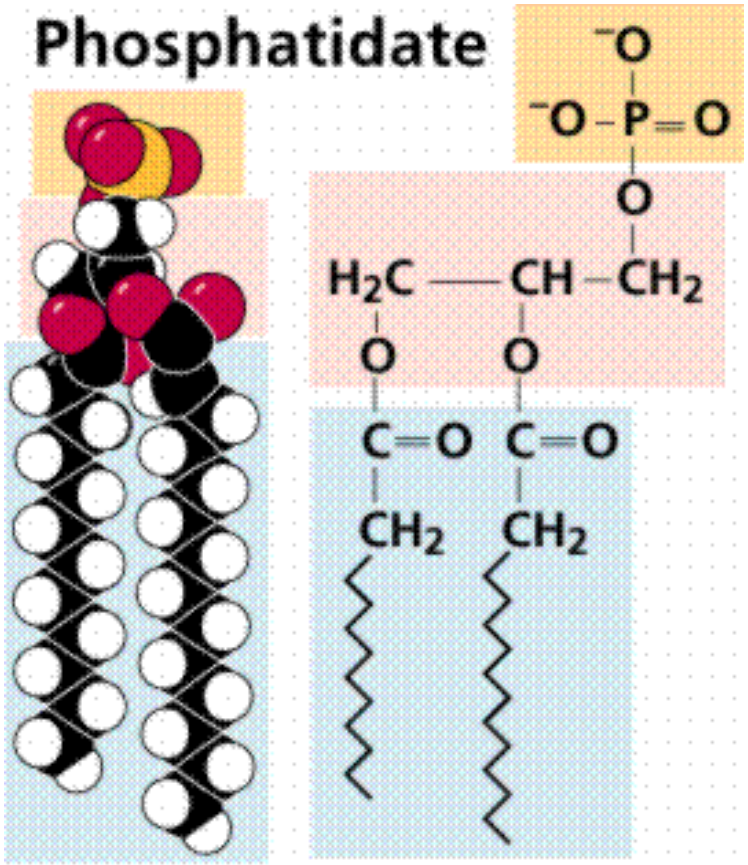
Therapeutic drugs



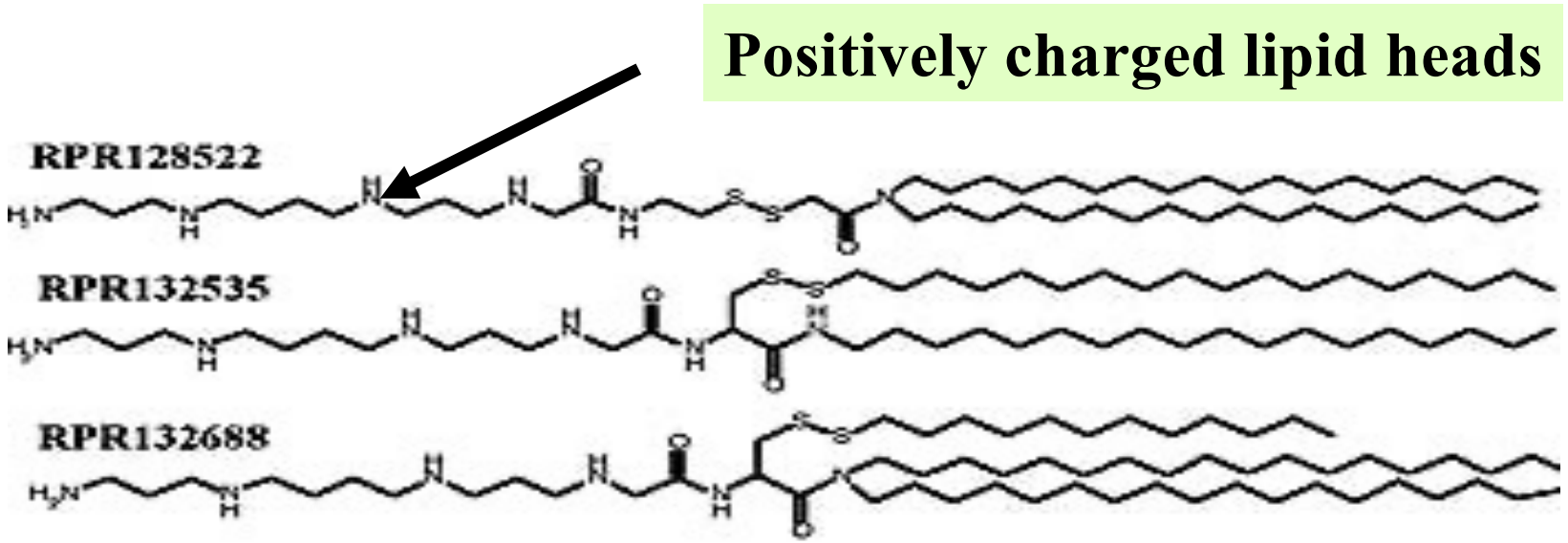
Liposomes are formed by the self-assembly of amphipathic molecules in an aqueous environment.

Anionic liposome





Cationic liposomes

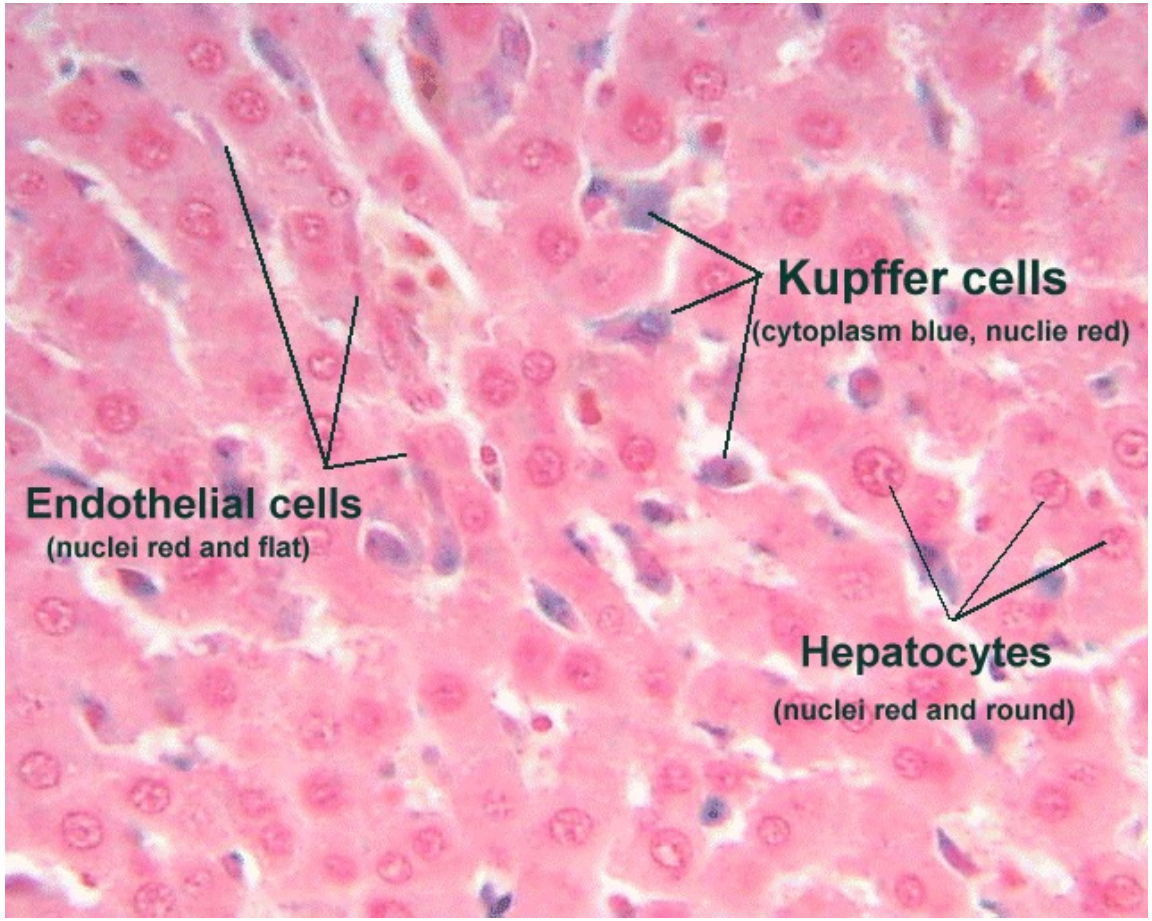


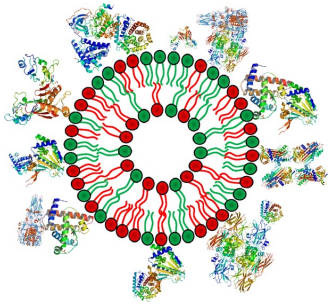
positively charged (amino groups) lipid droplets
can interact with negatively charged DNA
to wrap it up and deliver to cells

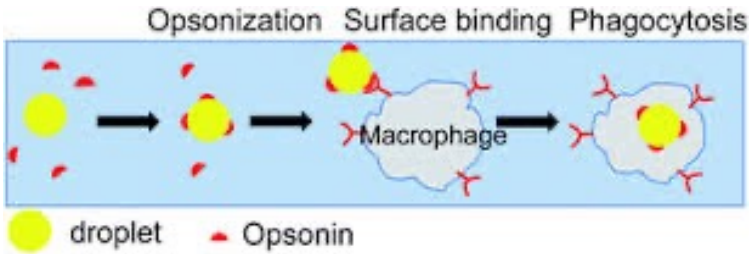
Inside liposomes, DNA is resistant to degradation

Short half life in vivo

Liposomes are rapidly cleared from the circulation
and largely taken up by the liver macrophages, after they are coated by plasma proteins **(opsonization)**





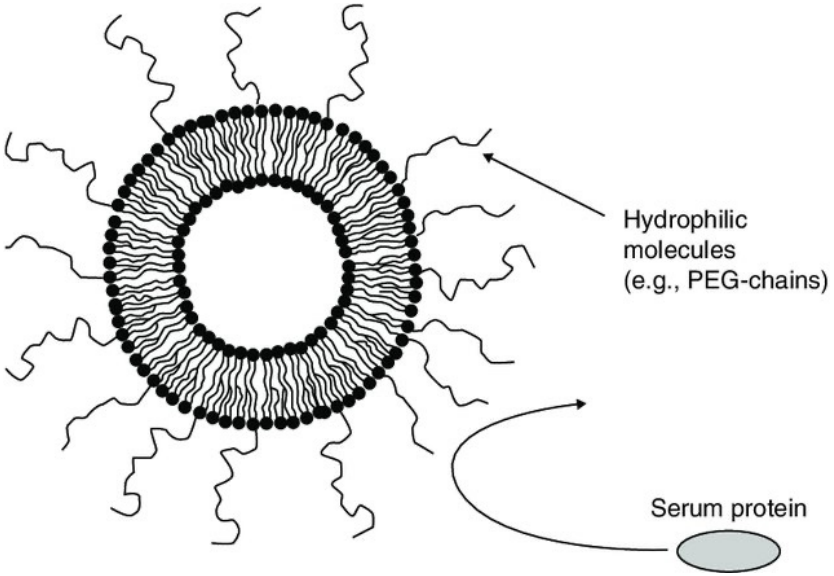


Kupffer cells
= liver macrophages

How to overcome it?

Modified liposomes (stealth liposomes)

hydrophilic polyethylene glycol (PEG), incorporated into liposome

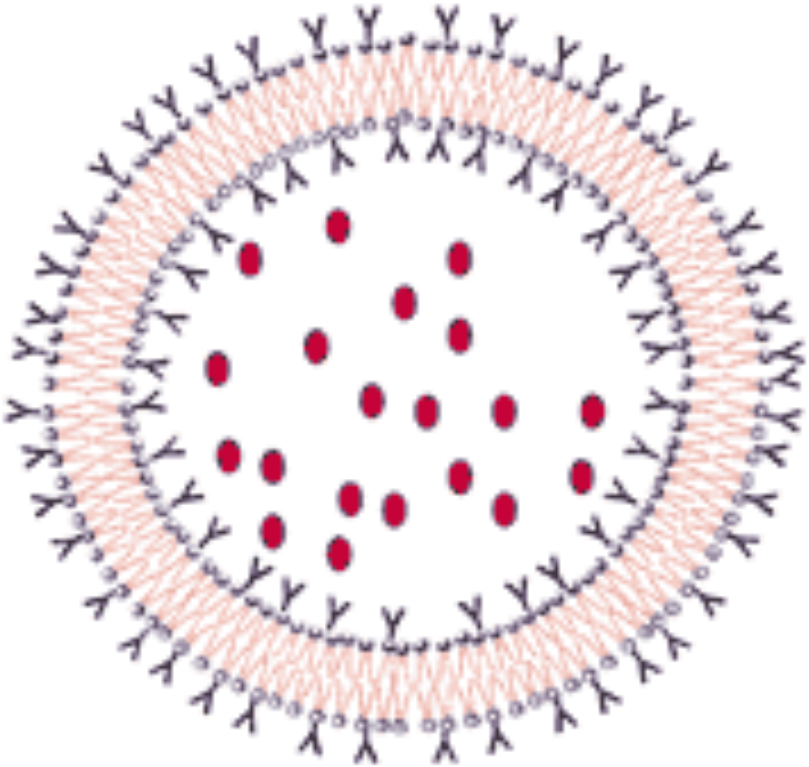


Increased half-life is due to
a **reduced coating (opsonisation)**
of these liposomes by plasma proteins

Cholesterol, polyvinyl-pyrrolidone polyacrylamide lipids, glucoronic acid lipids are working the same….

Liver cells not able to uptake them

Immunoliposomes for active targeting



examples

Antibodies to **intracellular myosin**
target liposomes
to the **heart (**infarcted areas)

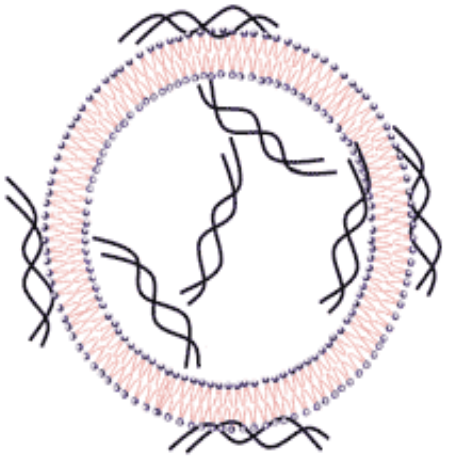
Antibodies against
tumor specific antigens
will target them to tumors

Advantages of liposomes

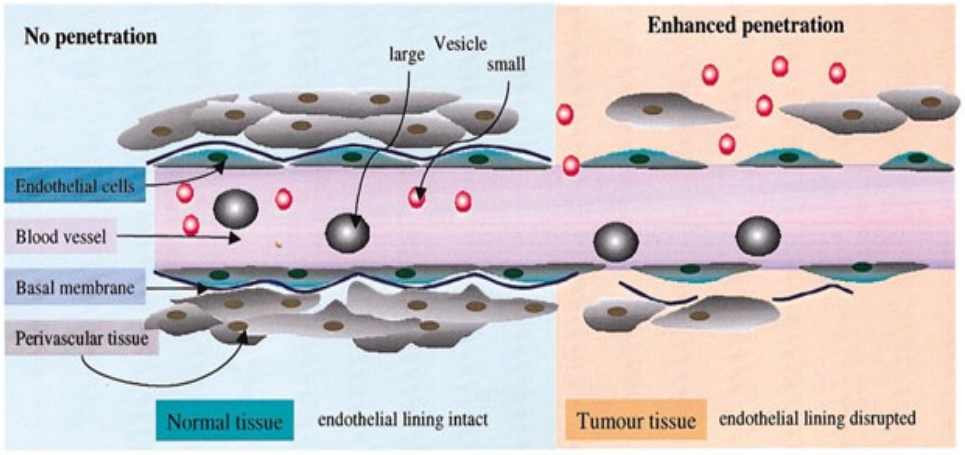
Cheaper than viruses

No immune response

100-1000 times more plasmid DNA needed for the same transfer efficiency as for viral vector



Could serve as **tumor specific vehicles**
(even without special targeting): better penetrate into tissues with disrupted endothelial lining

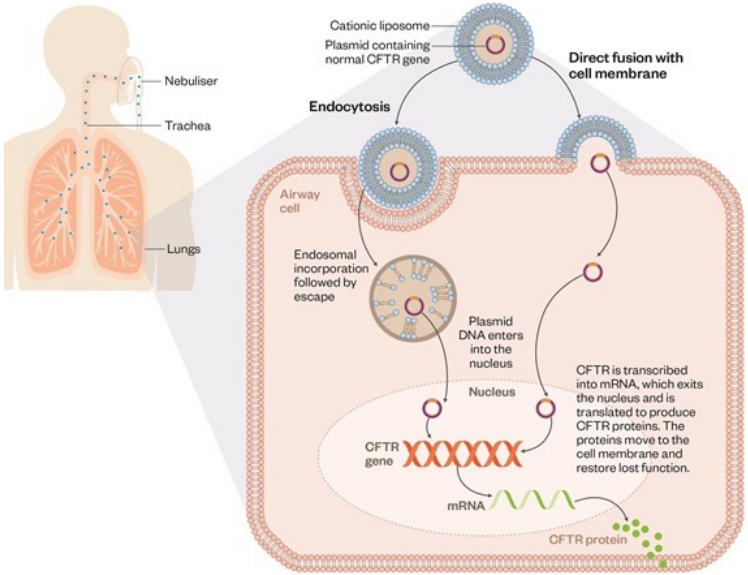


In vivo GT of CF with liposomes

The UK Cystic Fibrosis Gene Therapy Consortium

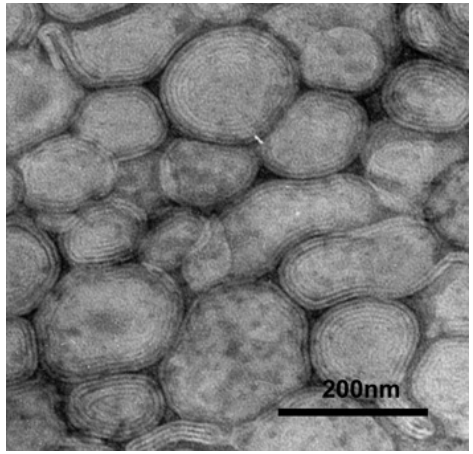
- http://www.cfgenetherapy.org.uk/

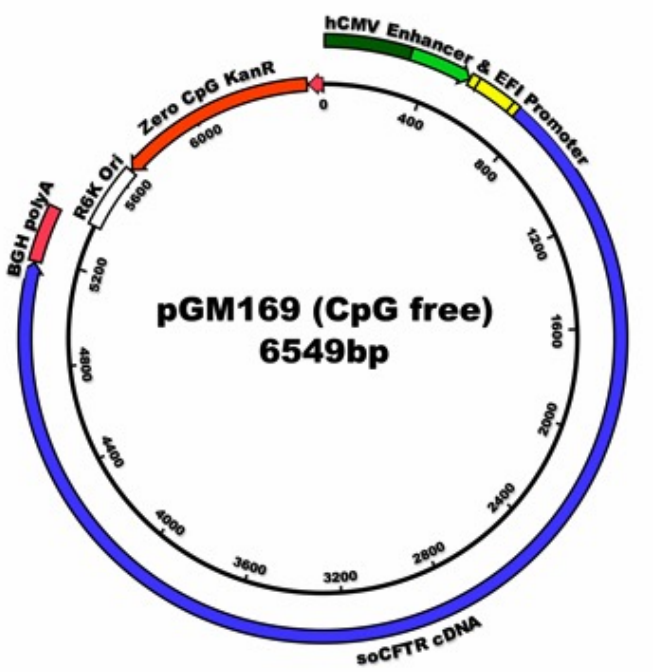
- A GT consortium, funded in 2001 in the UK that is experimenting an approach based on **liposomes administered by a nebulizer or nasal inhalation** to cure FC
  - University of Oxford,
  - University of London
  - University of Edinburgh.

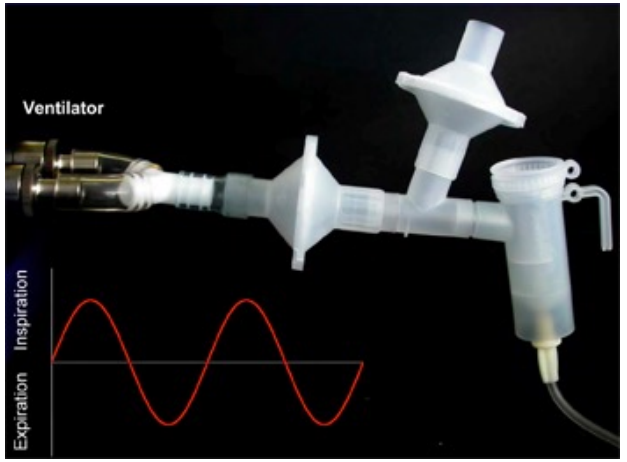


GL67A/pGM169

- **GL67A**: cationic liposome
- **pGM169**: plasmid encoding CFTR.







- **Liposomes containing the CFTR gene dissolved in saline and administered by single 5 ml dose every 28 days** per aerosolization for one year

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

Eric W F W Alton, David K Armstrong, Deborah Ashby, Katie J Bayfield, Diana Bilton, Emily V Bloomfield, A Christopher Boyd*, June Brand, Ruaridh Buchan, Roberto Calcedo, Paula Carvelli, Mario Chan, Seng H Cheng, D David S Collie, Steve Cunningham*, Heather E Davidson, Gwyneth Davies, Jane C Davies*, Lee A Davies, Maria H Dewar, Ann Doherty, Jackie Donovan, Natalie S Dwyer, Hala I Elgmati, Rosanna F Featherstone, Jemyr Gavino, Sabrina Gea-Sorli, Duncan M Geddes, James S R Gibson, Deborah R Gill*, Andrew P Greening, Uta Griesenbach*, David M Hansell, Katharine Harman, Tracy E Higgins*, Samantha L Hodges, Stephen C Hyde*, Laura Hyndman, J Alastair Innes*, Joseph Jacob, Nancy Jones, Brian F Keogh, Maria P Limberis, Paul Lloyd-Evans, Alan W Maclean, Michelle C Manvell, Dominique McCormick, Michael McGovern, Gerry McLachlan, Cuixiang Meng, M Angeles Montero, Hazel Milligan, Laura J Moyce, Gordon D Murray*, Andrew G Nicholson, Tina Osadolor, Javier Parra-Leiton, David J Porteous*, Ian A Pringle, Emma K Punch, Kamila M Pytel, Alexandra L Quittner, Gina Rivellini, Clare J Saunders, Ronald K Scheule, Sarah Sheard, Nicholas J Simmonds, Keith Smith, Stephen N Smith, Najwa Soussi, Samia Soussi, Emma J Spearing, Barbara J Stevenson, Stephanie G Sumner-Jones, Minna Turkkila, Rosa P Ureta, Michael D Waller, Marguerite Y Wasowicz, James M Wilson, Paul Wolstenholme-Hogg, on behalf of the UK Cystic Fibrosis Gene Therapy Consortium*

Lancet Respir Med 2015
Published Online July 3, 2015
http://dx.doi.org/10.1016/S2213-2600(15)00245-3

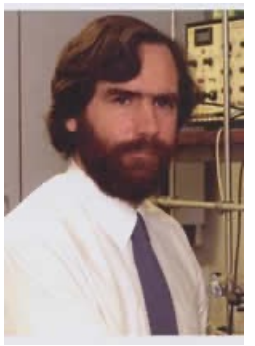
Summary

Background Lung delivery of plasmid DNA encoding the *CFTR* gene complexed with a cationic liposome is a potential treatment option for patients with cystic fibrosis. We aimed to assess the efficacy of non-viral *CFTR* gene therapy in patients with cystic fibrosis.

Methods We did this randomised, double-blind, placebo-controlled, phase 2b trial in two cystic fibrosis centres with patients recruited from 18 sites in the UK. Patients (aged ≥12 years) with a forced expiratory volume in 1 s (FEV_1) of 50–90% predicted and any combination of *CFTR* mutations, were randomly assigned, via a computer-based randomisation system, to receive 5 mL of either nebulised pGM169/GL67A gene–liposome complex or 0·9% saline (placebo) every 28 days (plus or minus 5 days) for 1 year. Randomisation was stratified by % predicted FEV_1 (<70 *vs* ≥70%), age (<18 *vs* ≥18 years), inclusion in the mechanistic substudy, and dosing site (London or Edinburgh). Participants and investigators were masked to treatment allocation. The primary endpoint was the relative change in % predicted FEV_1. The primary analysis was per protocol. This trial is registered with ClinicalTrials.gov, number NCT01621867.

Findings Between June 12, 2012, and June 24, 2013, we randomly assigned 140 patients to receive placebo (n=62) or pGM169/GL67A (n=78), of whom 116 (83%) patients comprised the per-protocol population. We noted a significant, albeit modest, treatment effect in the pGM169/GL67A group versus placebo at 12 months' follow-up (3·7%, 95% CI 0·1–7·3; p=0·046). This outcome was associated with a stabilisation of lung function in the pGM169/GL67A group compared with a decline in the placebo group. We recorded no significant difference in treatment-attributable adverse events between groups.

Interpretation Monthly application of the pGM169/GL67A gene therapy formulation was associated with a significant, albeit modest, benefit in FEV_1 compared with placebo at 1 year, indicating a stabilisation of lung function in the treatment group. Further improvements in efficacy and consistency of response to the current formulation are needed before gene therapy is suitable for clinical care; however, our findings should also encourage the rapid introduction of more potent gene transfer vectors into early phase trials.



Dr. Eric Alton

Fev = Forced Expiratory Volume

NEW TRIAL: nebulized CFTR Lentiviruses

UK Respiratory Gene Therapy Consortium

NEWS: Tuesday, 19th October 2021

Boehringer Ingelheim and Partners to Accelerate Development of First-In-Class Gene Therapy for Patients with Cystic Fibrosis

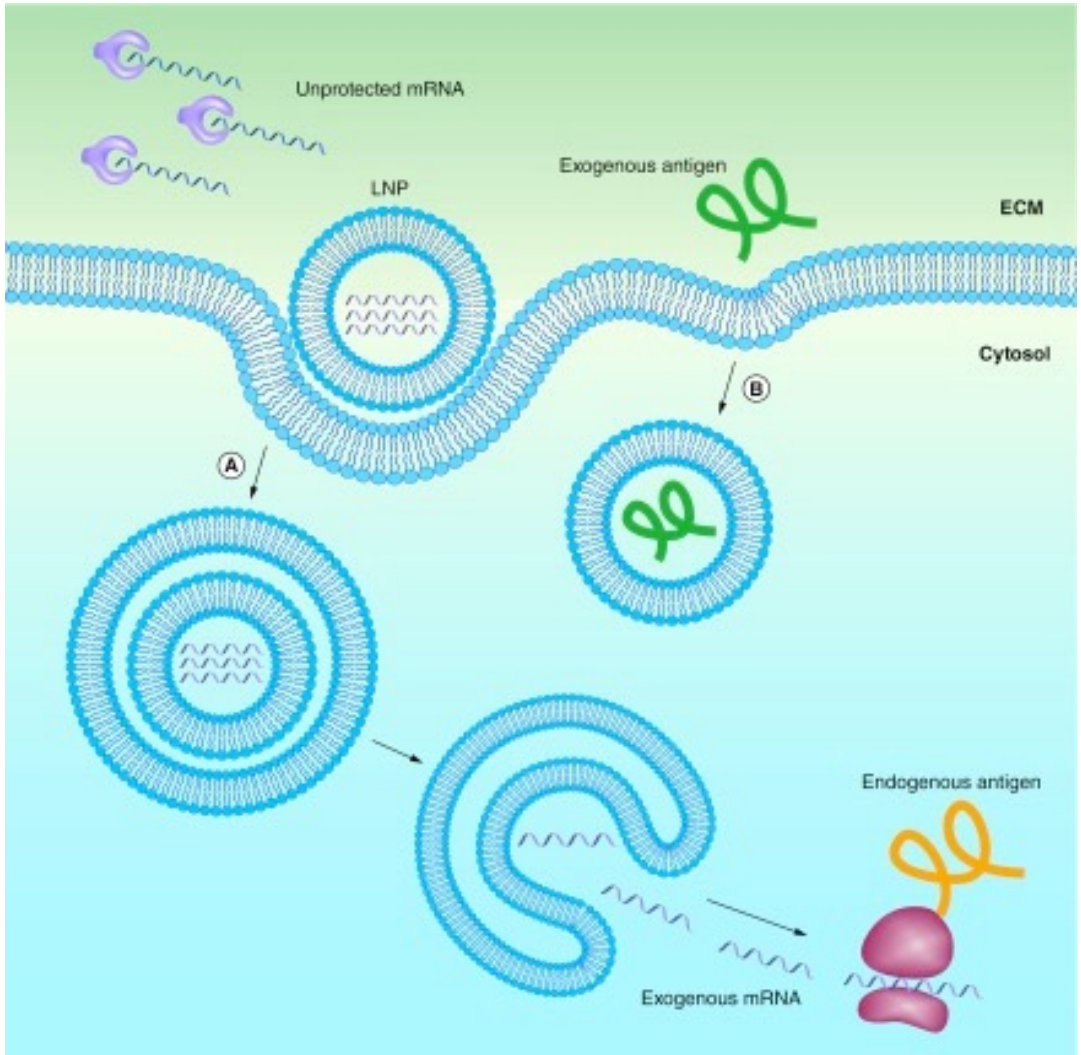
- *Boehringer Ingelheim has exercised intellectual property options from IP Group regarding research results generated by the UK Cystic Fibrosis Gene Therapy Consortium, and from Oxford Biomedica regarding their lentiviral vector technology*
- *Partners aim to expedite the development of the novel, inhaled cystic fibrosis transmembrane conductance regulator (CFTR) gene therapy BI 3720931 as a long-lasting therapeutic option for patients with cystic fibrosis (CF)*

Boehringer Ingelheim, IP Group, the UK Cystic Fibrosis Gene Therapy Consortium (GTC, consisting of researchers from Imperial College London and the Universities of Oxford and Edinburgh) and Oxford Biomedica (OXB), announced today that Boehringer Ingelheim has exercised its options on intellectual property and know-how from the partners to progress and further accelerate the development of a potential, new treatment option for patients with CF. In the partnership, IP Group, acting on behalf of the three GTC host Universities, is granting exclusive global rights to develop, manufacture, register, and commercialize this lentiviral vector-based gene therapy for the treatment of cystic fibrosis. The GTC is additionally contributing its knowledge in pre-clinical research and clinical gene therapy development. OXB is adding its leading competence in manufacturing lentiviral vector-based therapies to Boehringer Ingelheim's expertise in the development of novel breakthrough therapies for respiratory diseases.

Table 3
Clinical trials involving nucleic acids-loaded liposomes for the treatment of different syndroms.

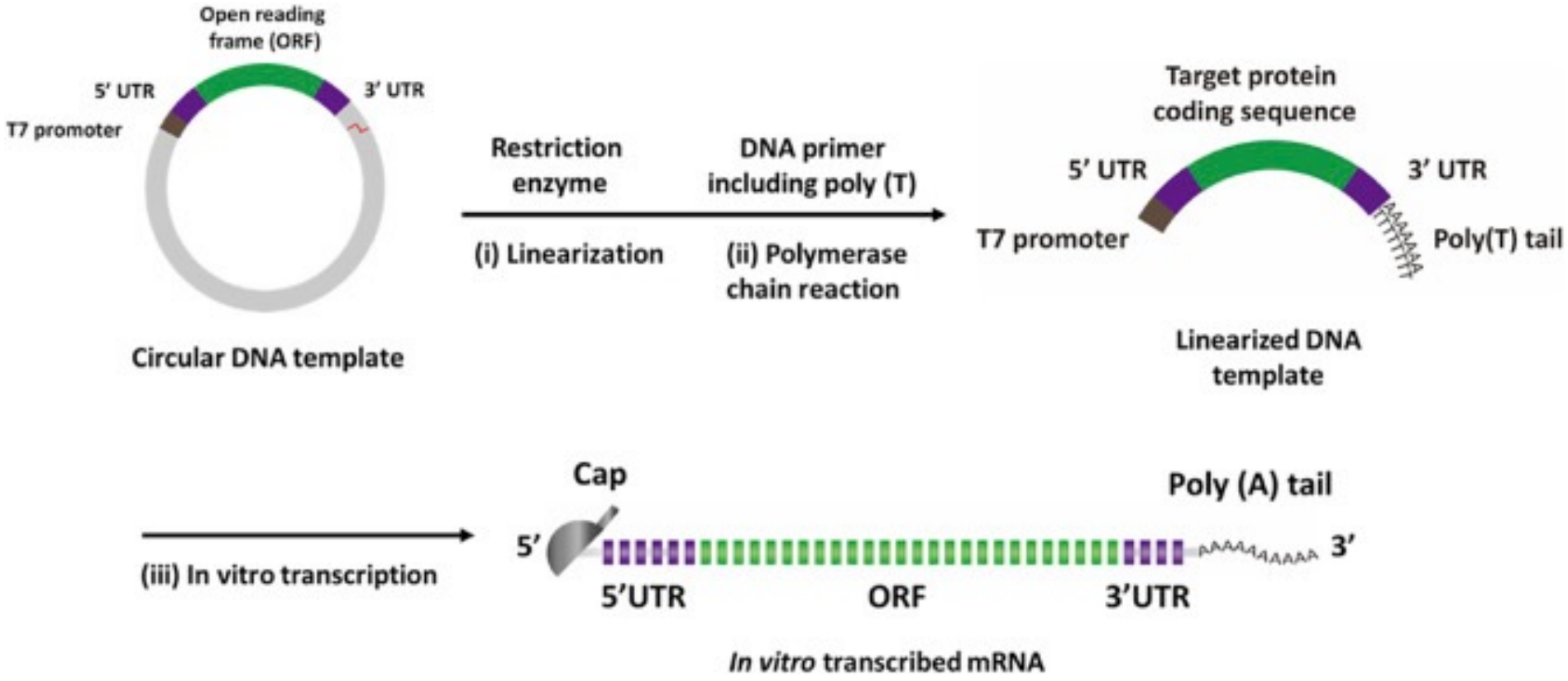
No.	Status	Study title	Condition	Interventions	Phase	Study reference
1	Recruiting	A Study of RNA-lipid Particle (RNA-LP) Vaccines.	Adult Glioblastoma	Autologous total tumor mRNA and LAMP mRNA	Phase 1	NCT04573140
2	Terminated	A Multicentre Phase I Study of MRX34, MicroRNA miR-RX34 Liposomal Injection	Primary Liver Cancer SCLC Lymphoma, Multiple Melanoma, Myeloma Renal Cell Carcinoma, NSCLC.	microRNA (MRX34)	Phase 1	NCT01829971
3	Recruiting	mRNA liposomal Vaccine in Combination With (Neo-)Adjuvant Chemotherapy (OLIVIA)	Ovarian Cancer	W_ova1 Vaccine	Phase 1	NCT04163094
4	Active	EphA2 siRNA in Treating Advanced or Recurrent Solid Tumors	Advanced Malignant Solid Neoplasm	EphA2-targeting DOPC-encapsulated siRNA	Phase 1	NCT01591356
5	Completed	Study to Determine Maximum Tolerated Dose of LErafAON Linked with Radiotherapy	Neoplasms	LErafAON	Phase 1	NCT00024648
6	Terminated	Evaluate the Safety, Tolerability, Pharmacokinetics (PK), and Pharmacodynamics (PD) of siRNA liposomes	Hypercholesterolemia	PRO-040201 and placebo	Phase 1	NCT00927459
7	Completed	Study With Atu027 in Patients with Advanced Solid Cancer	Advanced Solid Tumors	Atu027	Phase 1	NCT00938574
8	Completed	Study of Gene Therapy for Cystic Fibrosis	Cystic Fibrosis	pGT-1 gene lipid complex	Phase 1	NCT00004471
9	Completed	Interleukin-2 Gene or Methotrexate in Treating Patients with Recurrent or Refractory Stage III and IV Head and Neck Cancer	Head and Neck Cancer	Biological: gene therapy Biological: interleukin-2 gene Drug: methotrexate	Phase 2	NCT00006033
10	Completed	Phase I Study of IV DOTAP: CHOL -Fus1 in NSCLC	Lung Cancer	Genetic: DOTAP:Chol-fus1	Phase 1	NCT00059605
11	Recruiting	BP1001 in Pattern With Venetoclax Plus Decitabine in AML	Acute Myeloid Leukemia (AML)	Drug: BP1001 in combination with Ventoclax plus decitabine Drug: BP1001 plus decitabine	Phase 2	NCT02781883
12	Completed	BP1001 (L-Grb-2 Antisense Oligonucleotide) in CML, AML, ALL & MDS	CML, AML, ALL & MDS	Drug: BP1001 Drug: BP1001 in combination with LDAC	Phase 1	NCT01159028
13	Active	Cancer Vaccine to treat Advanced Melanoma (Lipo-MERIT)	Melanoma	Biological: Lipo-MERIT	Phase 1	NCT02410733
14	Recruiting	Autogene Cevumeran (RO7198457) as a Single Agent and in Sequence with Atezolizumab in Participants with Locally Advanced or Metastatic Tumors	Solid Cancers	Drug: Autogene cevumeran Drug: Atezolizumab	Phase 1	NCT03289962
15	Completed	Gene Therapy in CF Patients	Cystic Fibrosis	Drug: pGM169/GL67A Drug: Placebo	Phase 2	NCT01621867

Liposomes with mRNA

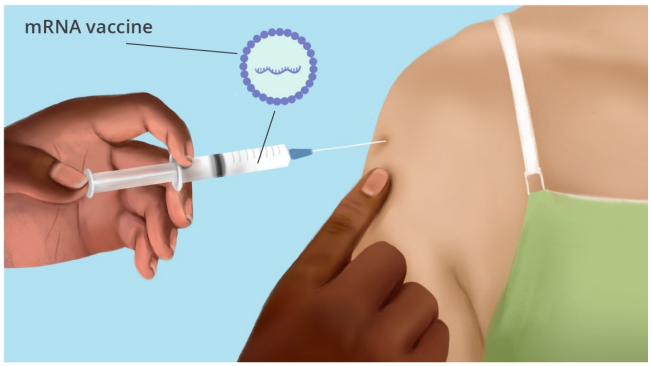


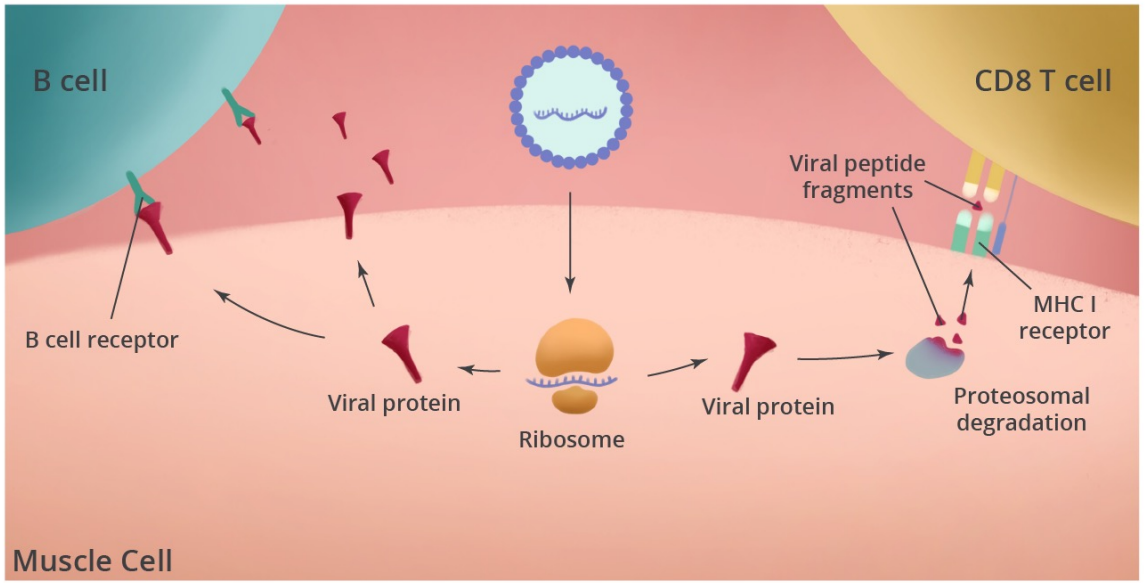
- In recent years, the use of mRNA instead of plasmids to express transgenes is emerging.
- mRNA does not need to enter the nucleus and be transcribed
- Less stable, therefore it needs to be conveyed: lipid nanoparticles (liposomes or solid nanoparticles)

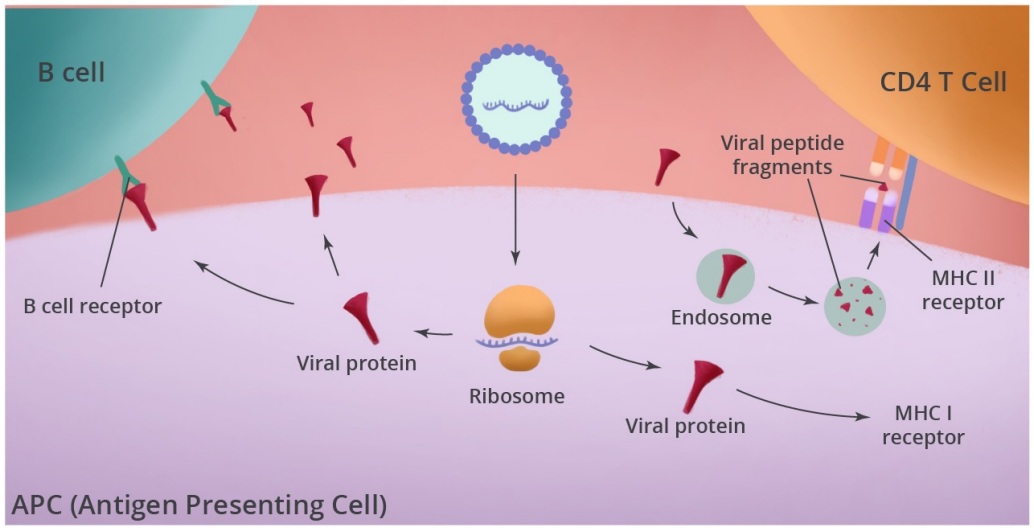
In vitro synthesis of mRNA



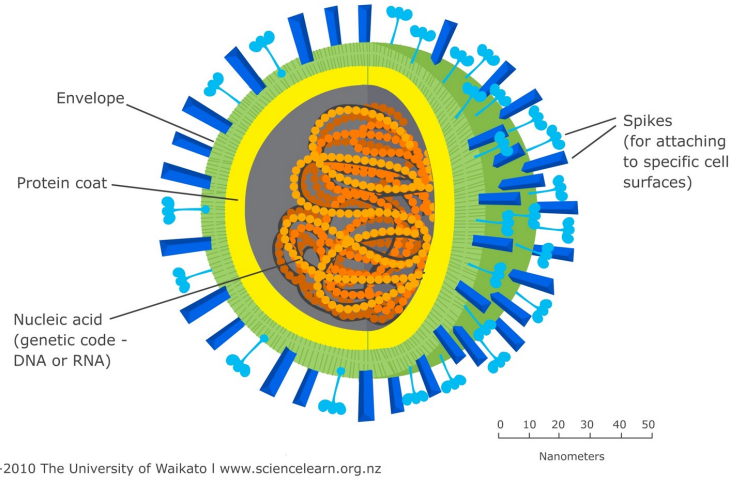
mRNA Vaccine: Comirnaty (Pfitzer) and Spikevax (Moderna)

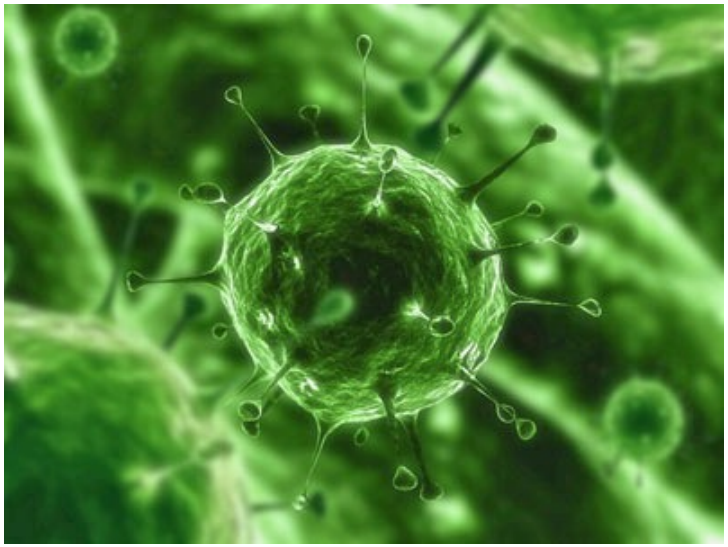






3-- GT with Viral Vectors





Benefits

- High **efficiency of transduction**
- Possibility of **integration**

Drawbacks

- May generate new dangerous viruses by **recombination** with viruses in the host
- **Insertional mutagenesis** (for those that randomly integrate in the genome)
- **Immunological reactions**
- May carry **DNA of limited length**
- **Expensive**

MOST COMMON VIRAL VECTORS

Retroviruses

can create double-stranded DNA copies of their RNA genomes. Can integrate into genome. MoMLV,

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

Lentiviruses

Similar to Retroviruses. Derived from HIV, may infect quiescent cells and integrate randomly.

Retrovirus

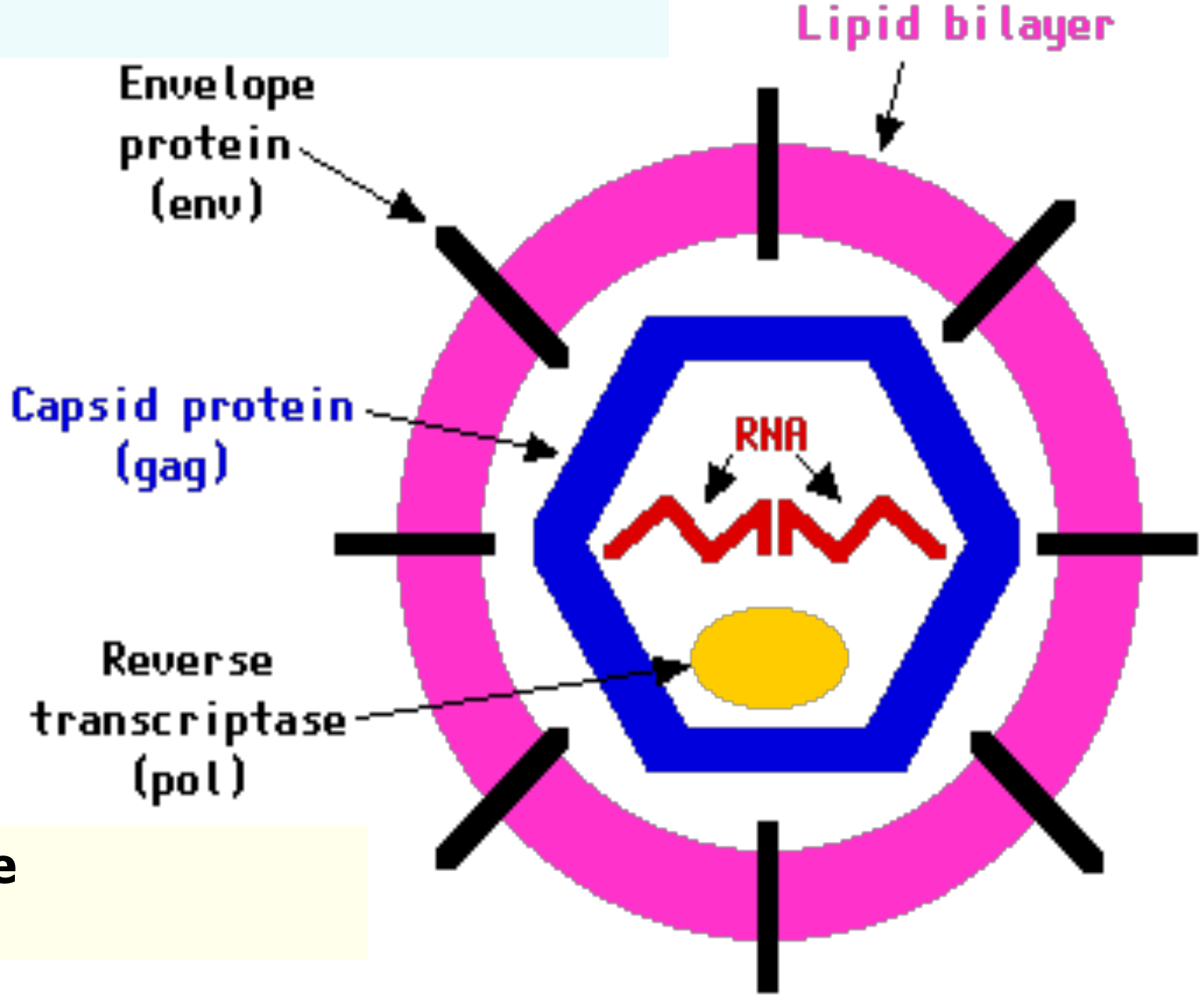
"minimal" retrovirus

Contains:

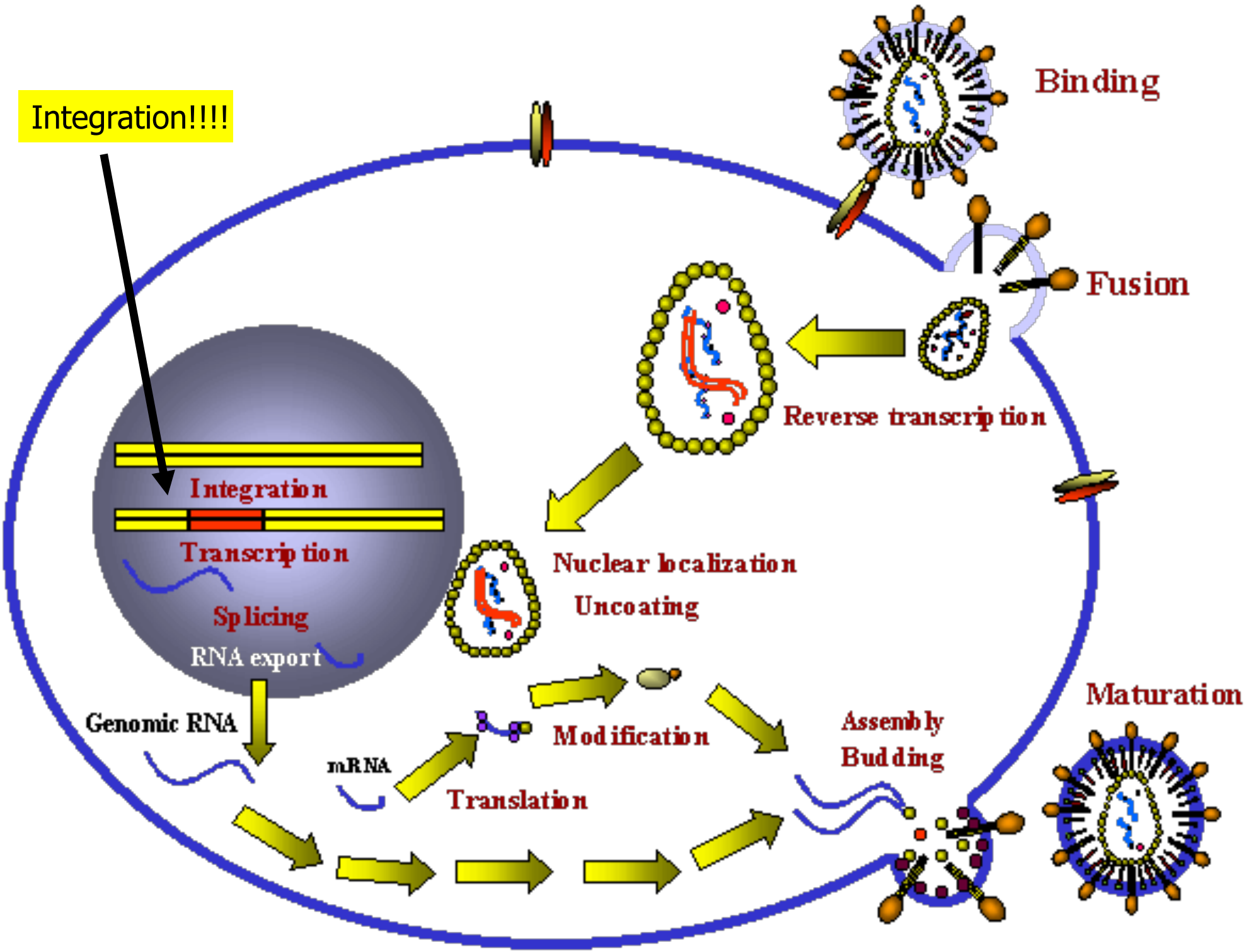
Env

Gag

Pol



POL = Reverse Transcriptase;
RNA→DNA


Integration!!!!
Binding
Fusion
Reverse transcription
Integration
Transcription
Splicing
RNA export
Nuclear localization
Uncoating
Genomic RNA
mRNA
Translation
Modification
Assembly
Budding
Maturation

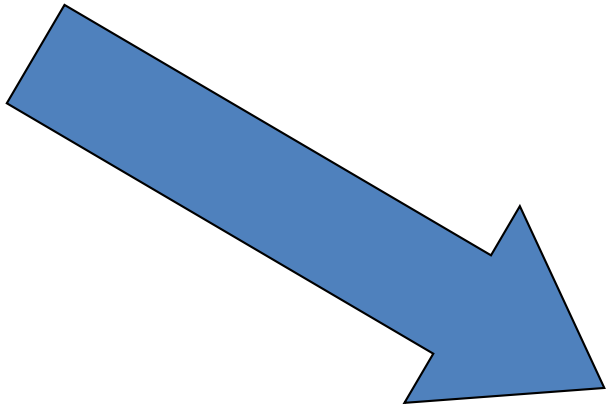
Retroviral Genome

capside, polimerase, envelope genes

Packing signal; Allows RNA encapsidation

Repeated Sequences: Contain a promoter and allow the insertion in the genome

For use in GT :
“attenuated” viruses, containing
“therapeutic gene”, packing sequences and repeated sequences



This virus goes to the patient
(unable to replicate)

Helper Virus– cannot pack (P);
Allows the replication of attenuated virus

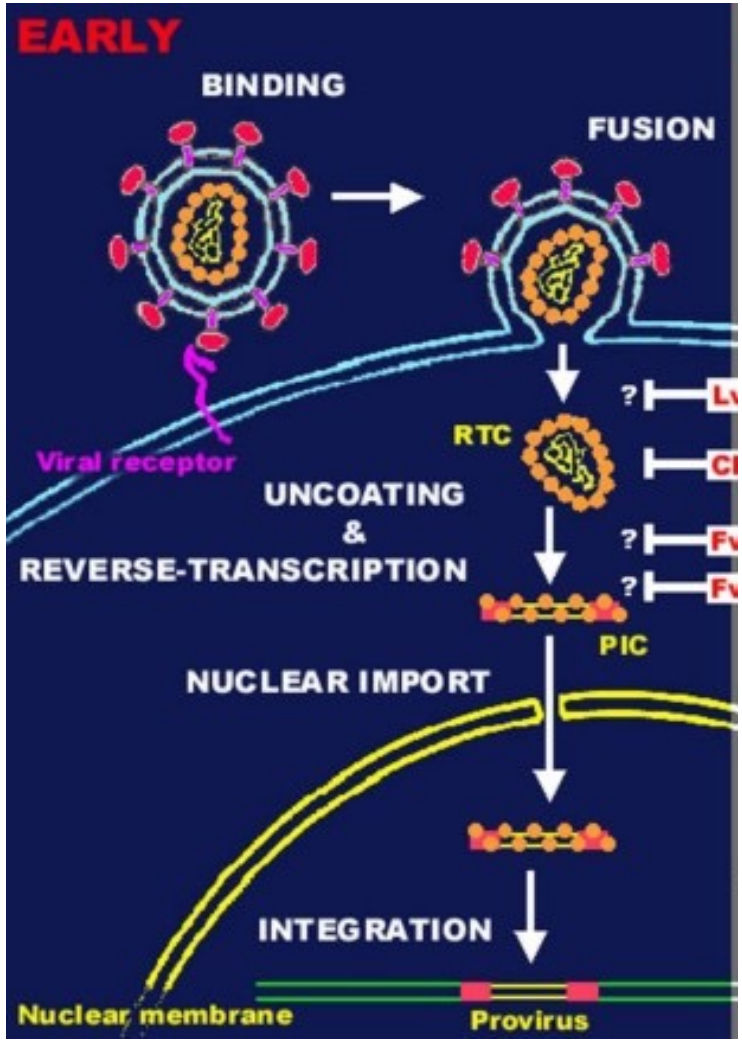
The helper virus
Is necessary to produce
the attenuated virus in cell culture

Retroviral vectors can infect dividing cells only

Preintegration complex (PIC) of retroviruses is not 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



Amphotropic retroviruses

Moloney murine leukaemia virus (**MMLV**),

capable of infecting both mouse cells and human cells

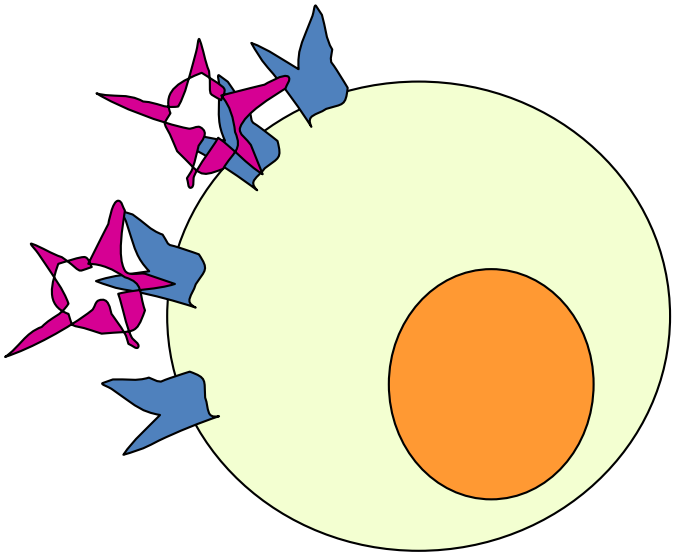
Treatment could be tested in mouse

After removing of all non-essential parts

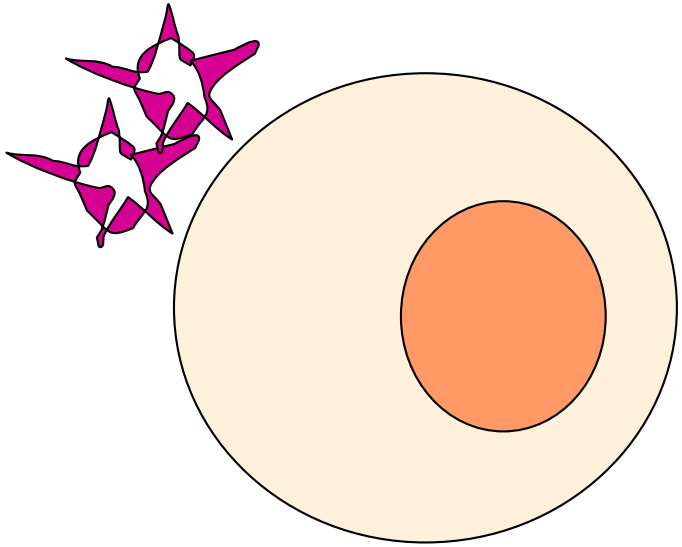
carrying capacity for retroviral vectors is approximately 7.5 kb

(not enough for some approaches)

Tissue tropism of retroviruses



In humans, retroviruses use
sodium-dependent phosphate transporters
Pit-1 and Pit-2 for entry



In humans
this receptor is expressed widely.

Treatments performed with retroviral systems

Severe Combined Immunodoficiency **(SCID):**
ADA-SCID and X-linked SCID

ADA gene therapy story

ADA protein has been characterized in the late 1970s

Three separate laboratories published the gene sequence in 1983

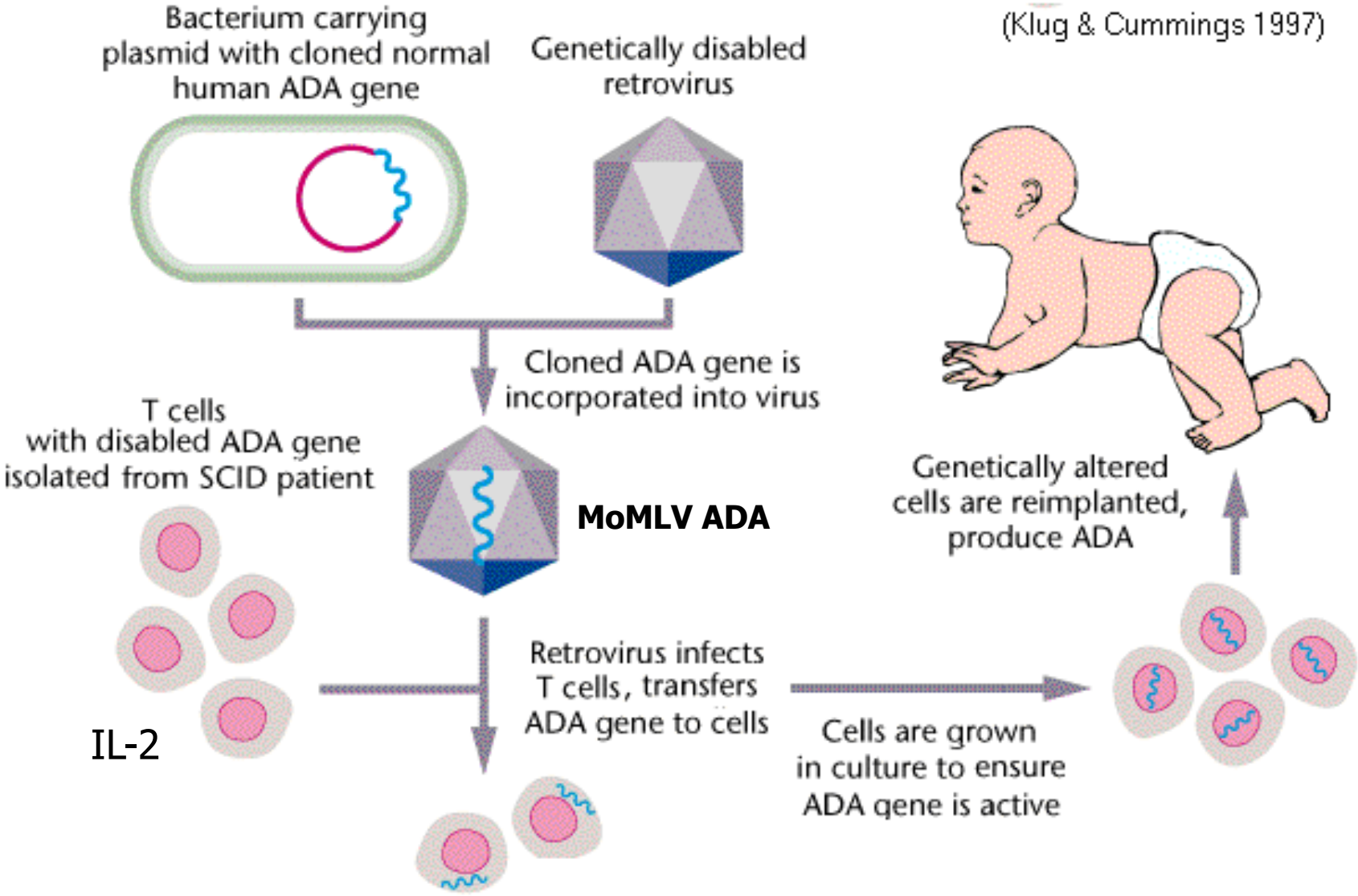
W. French Anderson (NIH); in the late summer of 1990, the FDA was sufficiently convinced by the preliminary laboratory data to approve the first human gene therapy trials using the MMLV-based delivery vector

September 14, 1990. **Mature T-cells GT**

Ashanti DeSilva; advanced stage of SCID; 4 yr old;

Cynthia Cutshall January 31, 1991

1st Clinical trial for ADA SCID (mature T cells)



Ex vivo GT of ADA SCID (Ashanti DeSilva)

There was a significant improvement although the injections had to be repeated

because T cells live
for only 6-12 months in the blood

The specific benefit of the gene therapy was unclear because she
continued to receive ADA-PEG

Science, 1995

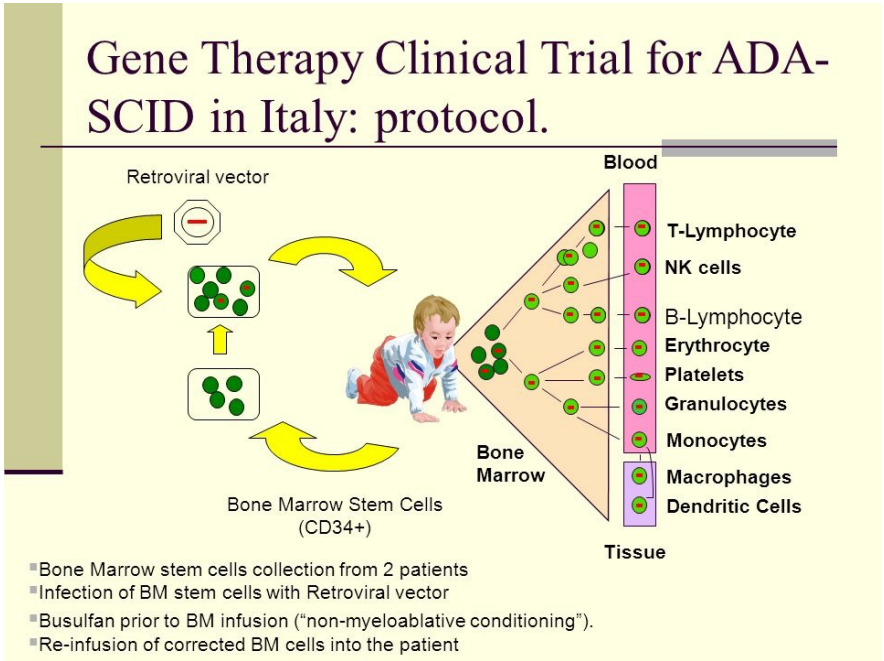
Next attempt: Hematopoietic Stem Cells (HSC)

Make more room for transgenic T cells by suppressing host bone marrow

2 patients with ADA SCID:

1) HSC **(CD 34+) infected with retro-ADA**.

2) Administration of **myelosuppressive drugs (busulfan) to block the growth of bone marrow** (but not to destroy it) to make 'space' for engineered marrow to implant, expand and grow better



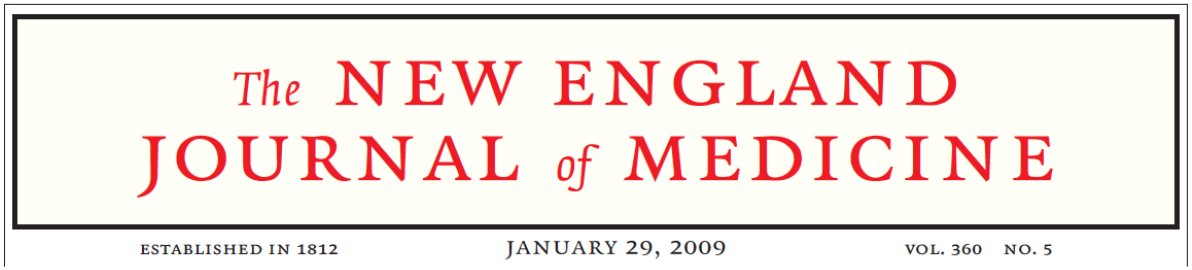
Results: amelioration of immunological functions (including antigen-specific response)

Reduction of toxic metabolites.

Both patients are currently at home and are healty.

Aiuti A et al., 2002 (*Science*)

Update of the protocol



Gene Therapy for Immunodeficiency Due to Adenosine Deaminase Deficiency

Group of **10 patients** treated with hematopoietic stem cells, transgenic for the ADA gene, implanted in mielosuppressive conditions.

After 4 years from the therapy, patient are all alive and healthy. Hematopoietic stem cells are stably implanted and are differentiatd into ADA-producing mieloid and lymphoid cells.

Conclusions: "GT associated to mielosuppression represents an unharmful and effective approach in patients with ADA SCID"

Approval! June 2016

Strimvelis®: approval by the European Medicines Agency (EMA). The first stem cell-based gene therapy protocol approved in the world!

X-Linked SCID: clinical trial

Dr Alain Fischer,
Necker Hopital, Paris
(1995-1997)

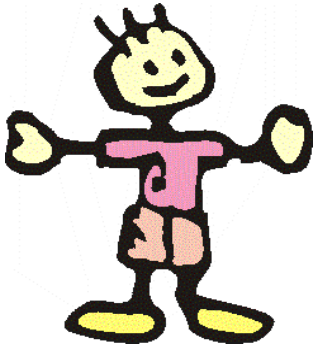
- **15 patients, 1-9 month of age**

- **Pick up of bone marrow , selection of CD34+ precursors**

- **3 daily exposure to the retroviral vector containing cDNA for the γc chain**

- **Reinfusion of bone marrow**

Results of X-SCID gene therapy



Alain Fischer at Necker Hospital, Paris

3,5 years after stem cells GT
This X-SCID children (14 out of 15)

- can **live normal lives at home** instead of inside a sterile "bubble";
- **have normal numbers of T cells** of both the CD4 and CD8 subsets;

- **have responded to several childhood immunizations**, including **diphtheria**, **tetanus** and **polio** by producing both T cells and antibodies specific for these agents.

- Antibody production is sufficiently good that they have **no need for periodic infusions of immunoglobulin** (IG).

Science. 2000 Apr 28;288(5466):669-72.

Leukemia in X-SCID treated patients

2002:

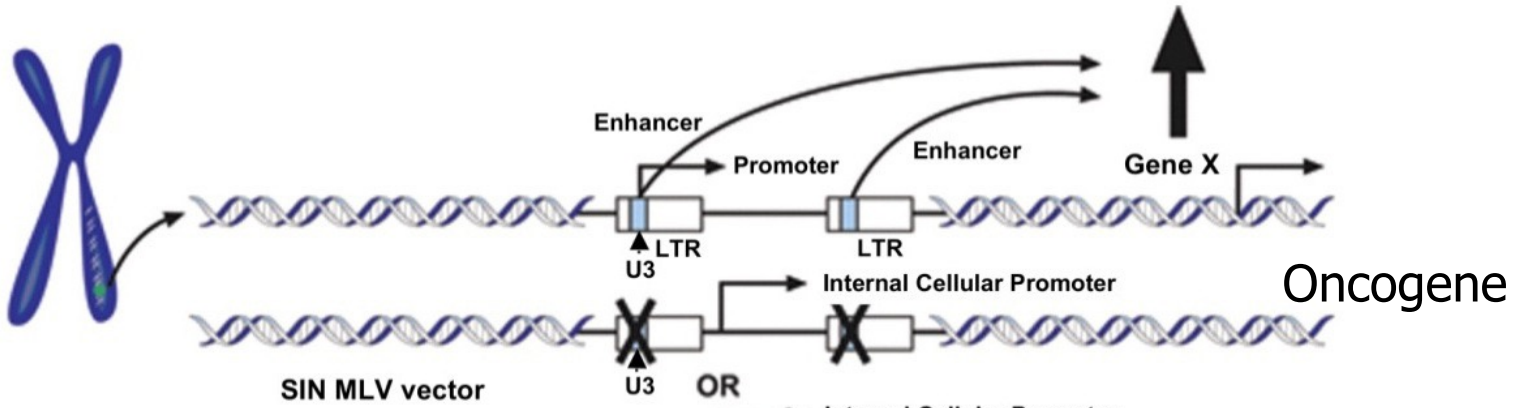
In **2 cases** therapeutic gene insert itself near the *LMO2* proto-oncogene

LMO2 = LIM domain Only 2 transcription regulator, plays a role in angiogenesis

Rearranged in T-ALL.
Transgenic mice with enforced expression of LMO2 in their thymocytes develop T cell leukemias…

The US Food and Drug Administration (FDA) halted 27 gene therapy trials

Update 2014:
Self inactivating SIN-γc retrovirus



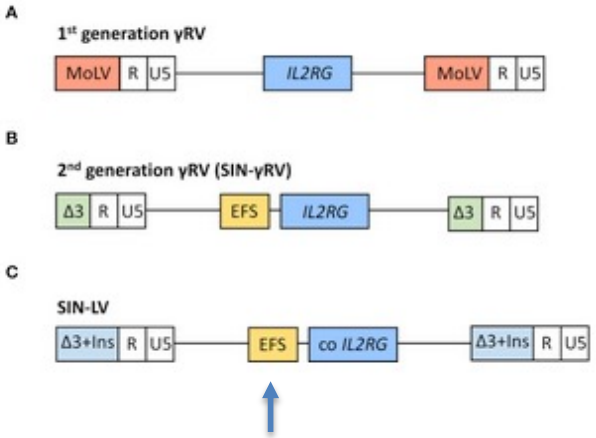


FIGURE 2 Schematic representation of viral vectors used in the different clinical trials for the treatment of SCID-X1 patients. In 1st generation γ retroviral vectors (γRVs), the gene expression is controlled by viral long terminal repeat (LTR) sequences (A). In 2nd generation self-inactivating (SIN) γRV and lentiviral vectors (LVs), the U3 region of LTR is deleted and the human interleukin-2 receptor γ gene (*IL2RG*) expression is driven by an internal mammalian promotor (B). Moreover, the LVs contain a codon optimized (co) *IL2RG* complementary DNA to further improve the transgene expression, and U3 region is replaced with a chromatin insulator element (Ins) (C). MoLV, Moloney murine leukemia virus; EFS, eukaryotic human elongation factor 1α (EF1α) short promoter.

A Modified γ-Retrovirus Vector for X-Linked Severe Combined Immunodeficiency

ABSTRACT

BACKGROUND

In previous clinical trials involving children with X-linked severe combined immunodeficiency (SCID-X1), a Moloney murine leukemia virus–based γ-retrovirus vector expressing interleukin-2 receptor γ-chain (γc) complementary DNA successfully restored immunity in most patients but resulted in vector-induced leukemia through enhancer-mediated mutagenesis in 25% of patients. We assessed the efficacy and safety of a self-inactivating retrovirus for the treatment of SCID-X1.

METHODS

We enrolled nine boys with SCID-X1 in parallel trials in Europe and the United States to evaluate treatment with a self-inactivating (SIN) γ-retrovirus vector containing deletions in viral enhancer sequences expressing γc (SIN-γc).

RESULTS

All patients received bone marrow–derived CD34+ cells transduced with the SIN-γc vector, without preparative conditioning. After 12.1 to 38.7 months of follow-up, eight of the nine children were still alive. One patient died from an overwhelming adenoviral infection before reconstitution with genetically modified T cells. Of the remaining eight patients, seven had recovery of peripheral-blood T cells that were functional and led to resolution of infections. The patients remained healthy thereafter. The kinetics of CD3+ T-cell recovery was not significantly different from that observed in previous trials. Assessment of insertion sites in peripheral blood from patients in the current trial as compared with those in previous trials revealed significantly less clustering of insertion sites within *LMO2*, *MECOM*, and other lymphoid proto-oncogenes in our patients.

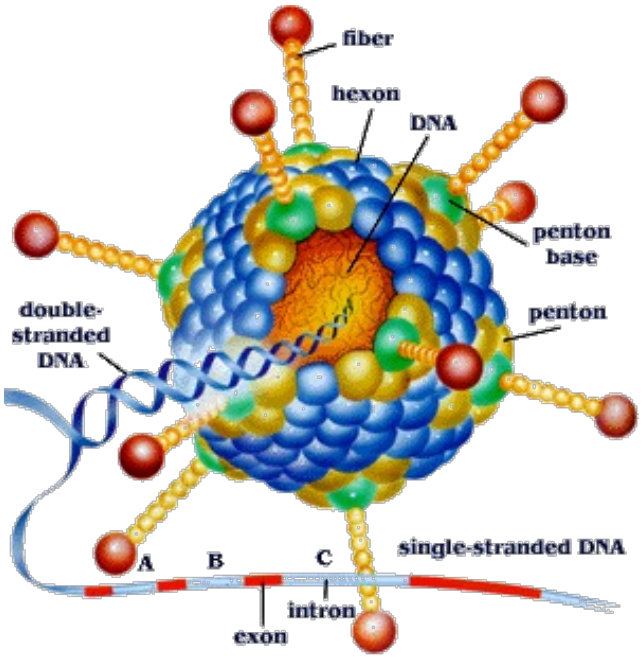
CONCLUSIONS

This modified γ-retrovirus vector was found to retain efficacy in the treatment of SCID-X1. The long-term effect of this therapy on leukemogenesis remains unknown. (Funded by the National Institutes of Health and others; ClinicalTrials.gov numbers, NCT01410019, NCT01175239, and NCT01129544.)

ADENOVIRUSES

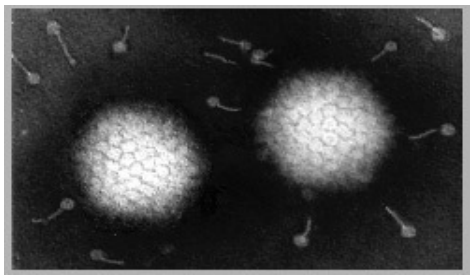
non-enveloped viruses
containing a linear double stranded DNA genome

40 serotypes known;
most causing respiratory infections in humans



www.nobel.se

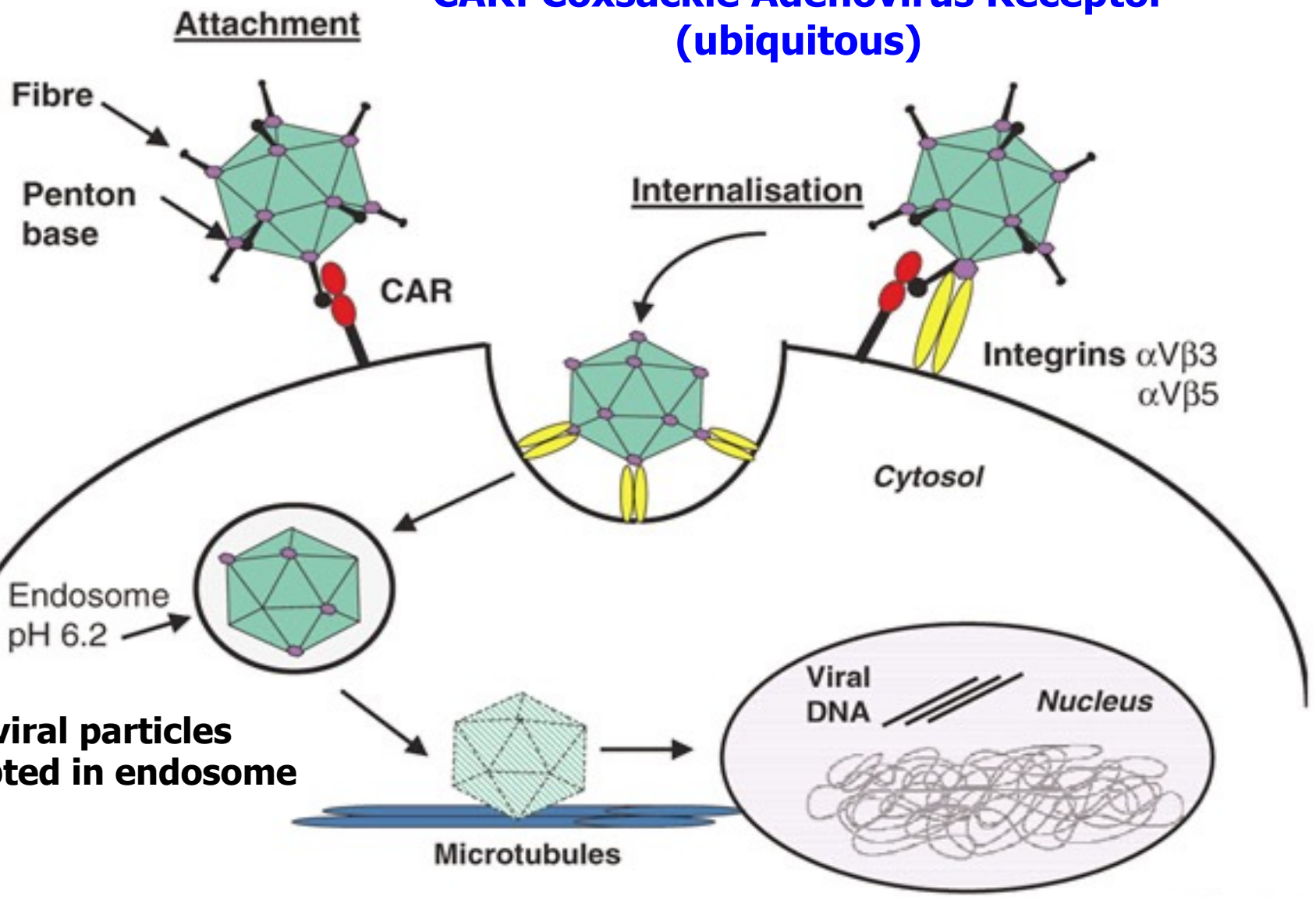
can infect both
dividing and nondividing cells



Icosahedral capside
12 antenna-like fiber projections
for virus attachment

Attachment via CAR, internalization via integrins

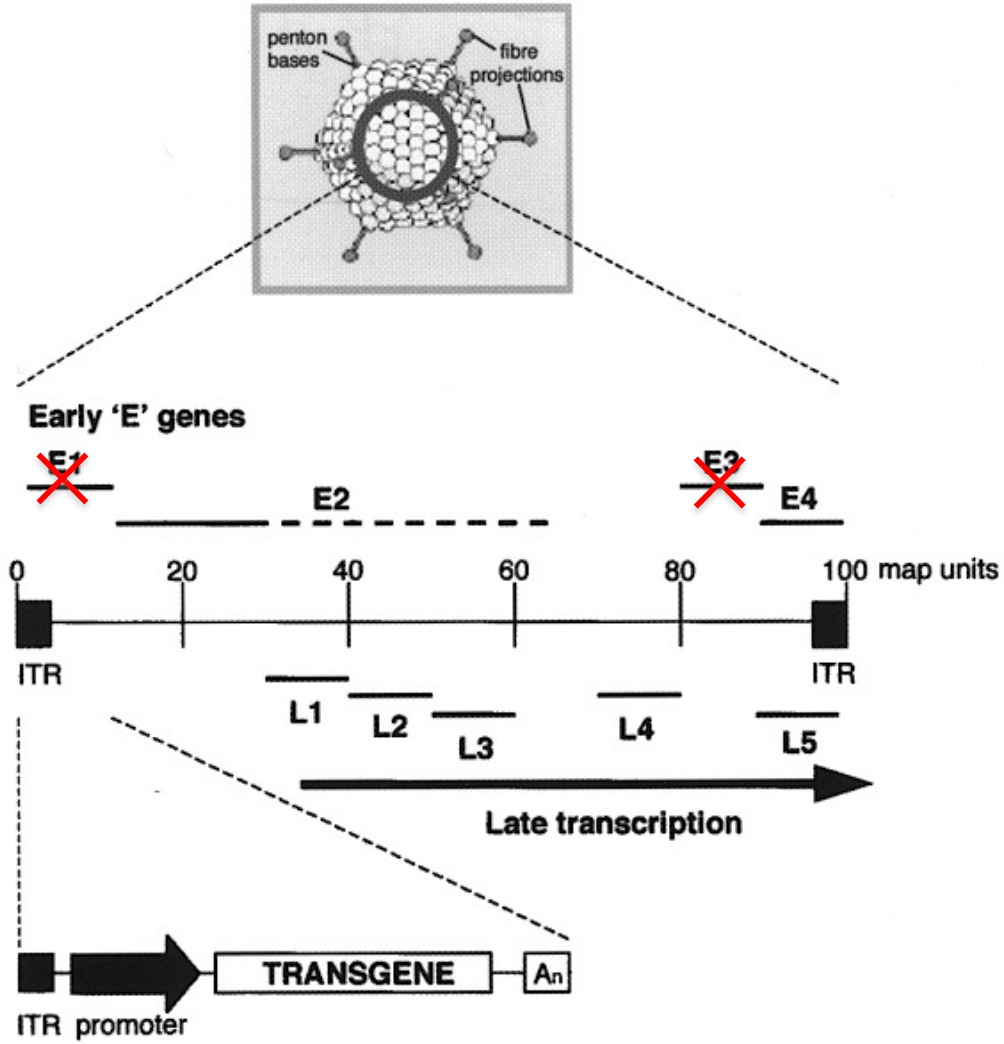
CAR: Coxsackie Adenovirus Receptor (ubiquitous)



Adenoviral particles are disrupted in endosome

Adenoviral vectors for gene therapy

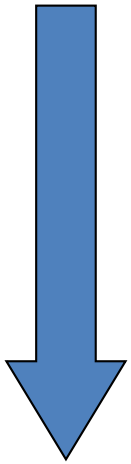
Adenoviral genome of 36 Kb contains over a dozen genes



Adenoviral vectors do NOT integrate

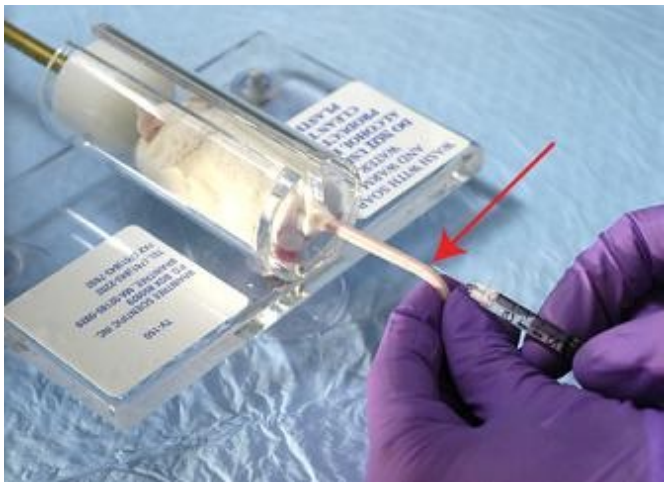
Any therapy based on adenoviral gene transfer would require repeated applications of the vector

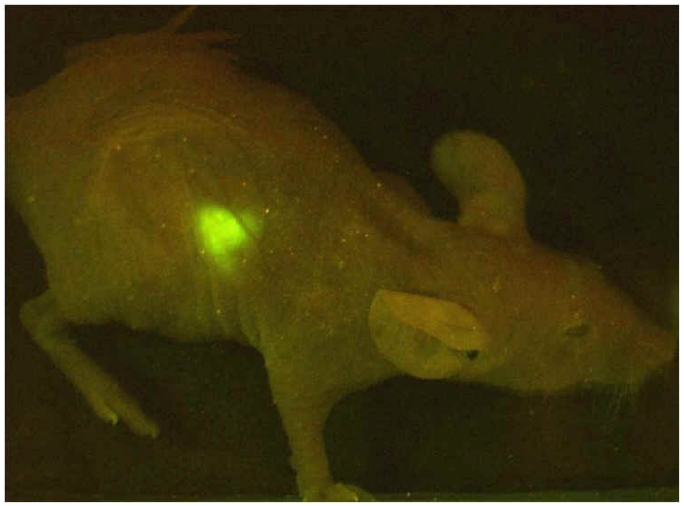
increased risk of recombination,
especially if wild type infection occur simultaneously



Risk of toxicity and immune responses

In vivo GT with adenoviruses: liver tropism

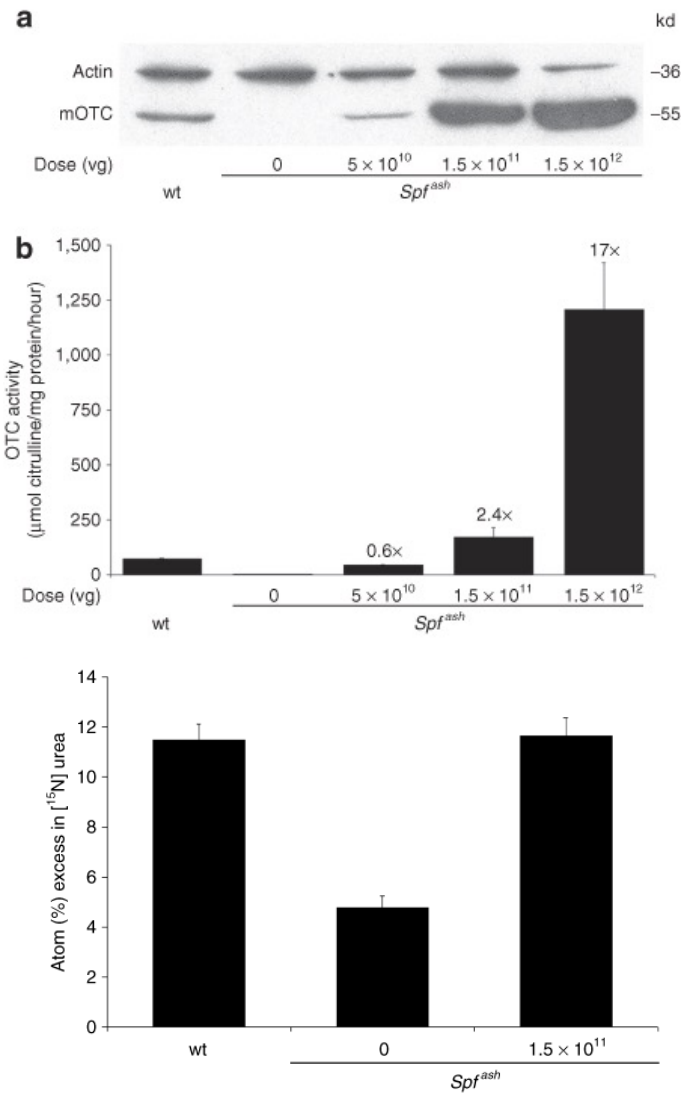




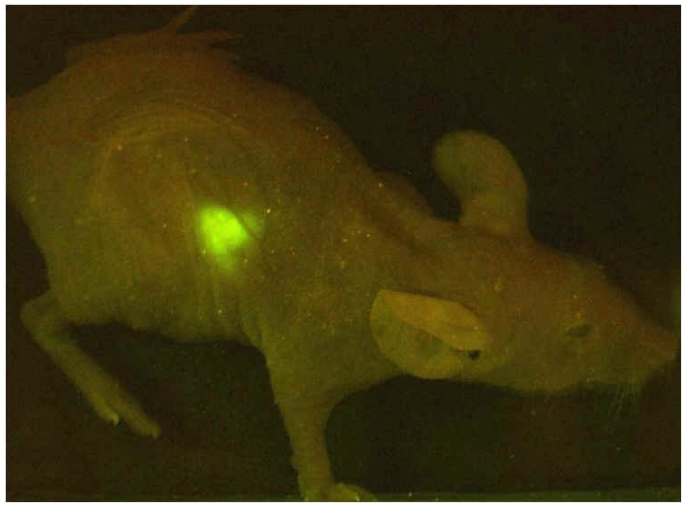
- Purified adenovirus may be injected in the mouse tail vein.
- It infects almost exclusively the liver.
- Ideal approach for GT targeting the liver (e.g. metabolic diseases).

In vivo GT of OTC deficiency:

mouse models (Spf^{ash})



GT with Ad-mOTC: excellent results in restoring OTC and circulating urea levels

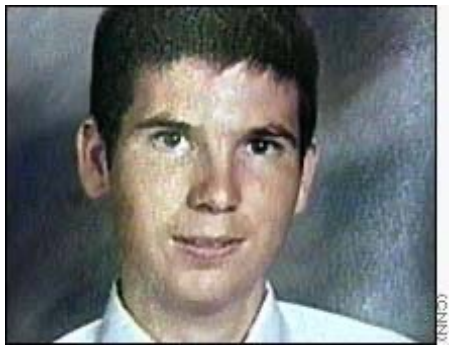


Human trial for OTC deficiency (Upenn 1999)

6 escalation doses i.h. ; up to 10^{13} at the dose level 6

third generation Ad-hOTC vector

NIH's National Gene Vector Laboratories' facility in UPenn



Jesse **Gelsinger** , an 18-year-old from Arizona
died after fast developing fever and organ failures

Cause of death:
massive immune reaction

After his death, all gene therapy trials in the United States **were halted**

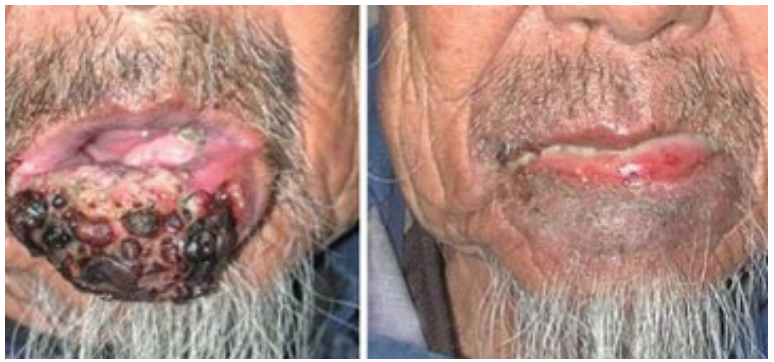
GENDICINE

(gene + medicine)

- **First drug in the world approved for cancer GT (Chinese FDA, in 2003)**

- **Used in the treatment of**
  - **Head and neck squamous cell carcinoma**
  - **lung carcinoma**

- **Adenovirus expressing recombinant p53, injected in the tumor**



Re-introduction of p53 in cancer cells

p53 does not have a toxic effect on normal cells but kills cancer cells

Bystander effect on non transgenic cells:

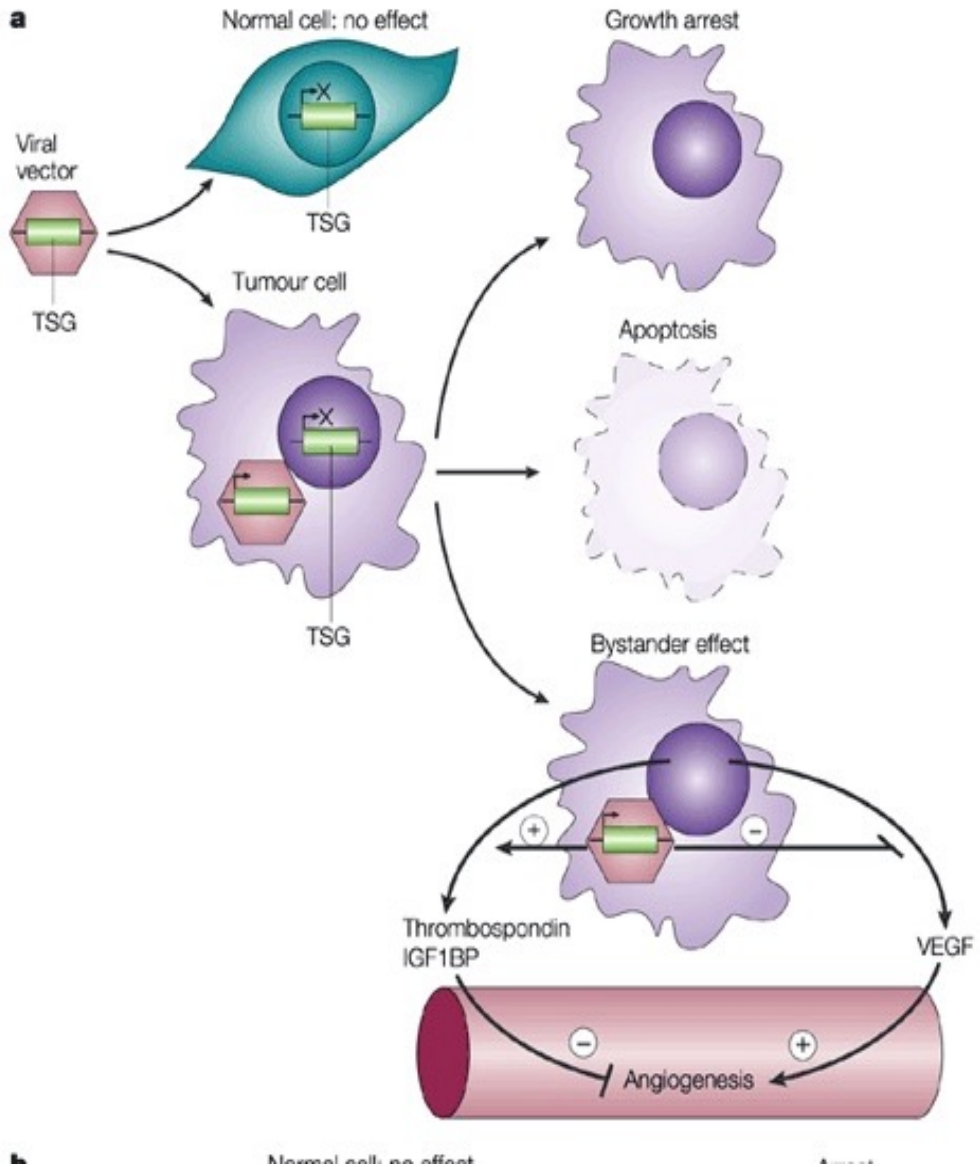
p53 blocks angiogenesis:

Downregulates VEGF production and

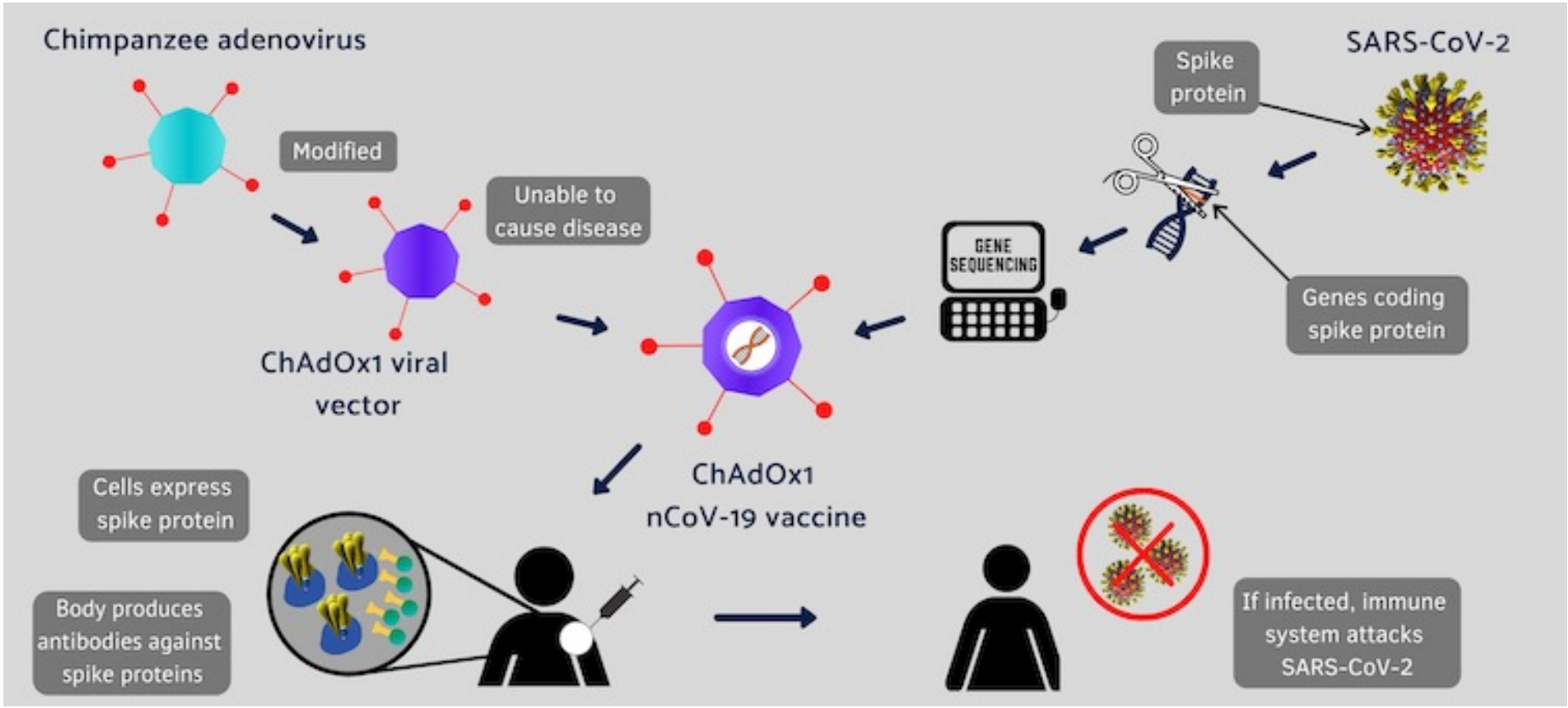
Upregolates two anti-angiogenic molecules:

1)Thrombospondin;

2) Insulin-like growth factor 1 binding protein (IGF1BP).



COVID-19 Vaccines
Astrazeneca/Johnson & Johnson



ADENO-ASSOCIATED VIRUS (AAV)

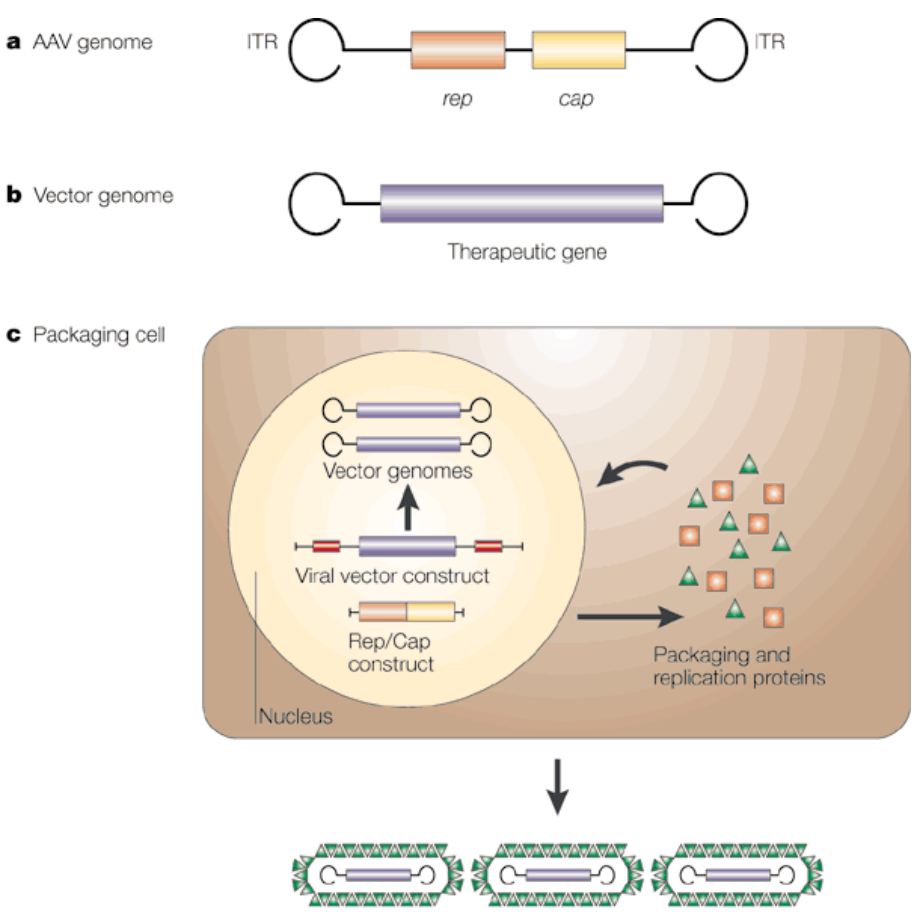
Single stranded DNA viruses, simple, **non-pathogenic** they require a helper virus (usually an adenovirus) to replicate.

Have two genes (**cap e rep**) located between two inverted repeats.

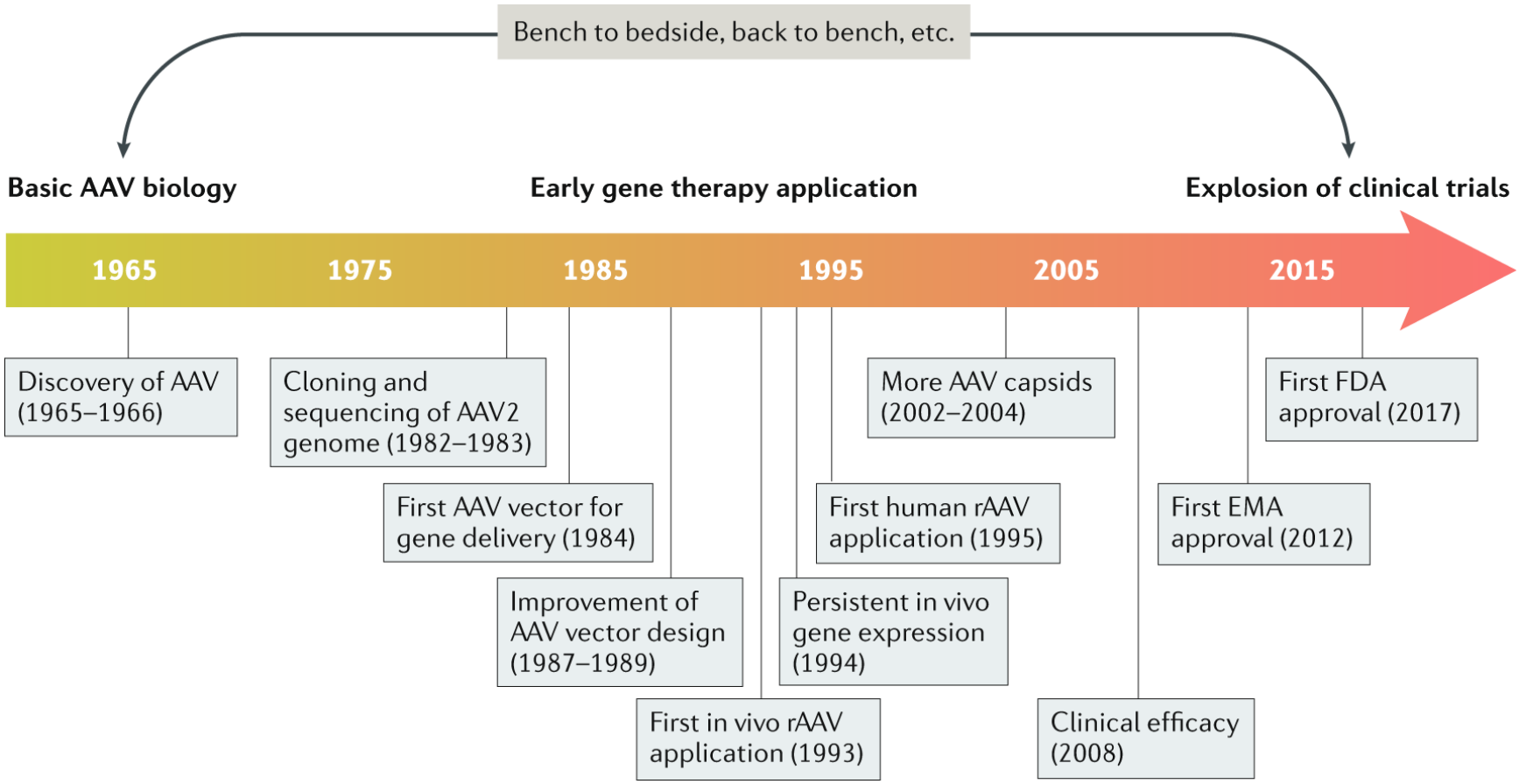
The **cap** gene encodes proteins of the viral **capside** while the **rep** gene for viral **replication and integration** proteins.

May infect many **cellular** types

The viral **DNA may integrate, into the host chromosome, but with very low efficiency.**



AAV in GT


Bench to bedside, back to bench, etc.
Basic AAV biology
Early gene therapy application
Explosion of clinical trials
1965
1975
1985
1995
2005
2015
Discovery of AAV (1965–1966)
Cloning and sequencing of AAV2 genome (1982–1983)
First AAV vector for gene delivery (1984)
Improvement of AAV vector design (1987–1989)
First in vivo rAAV application (1993)
Persistent in vivo gene expression (1994)
First human rAAV application (1995)
More AAV capsids (2002–2004)
Clinical efficacy (2008)
First EMA approval (2012)
First FDA approval (2017)

Adeno-associated virus

Pros:

Nonpathogenic

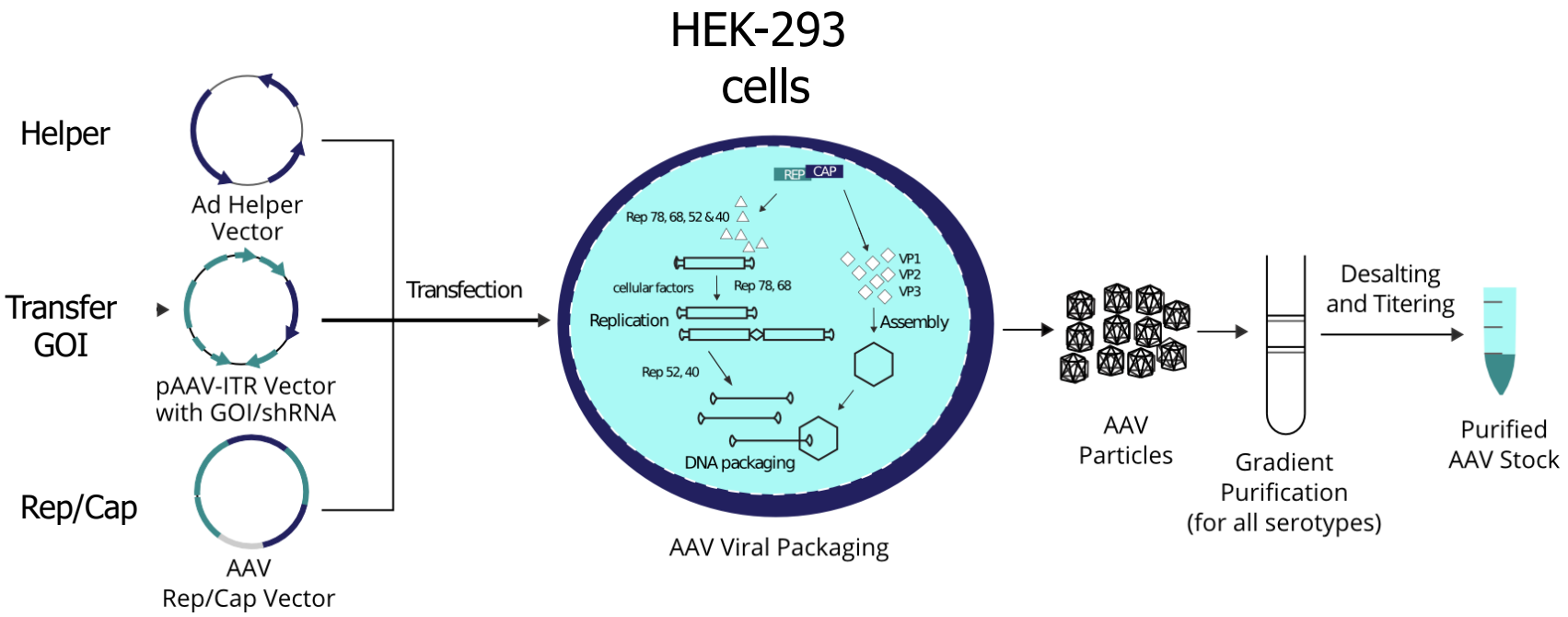
Infects non dividing cells

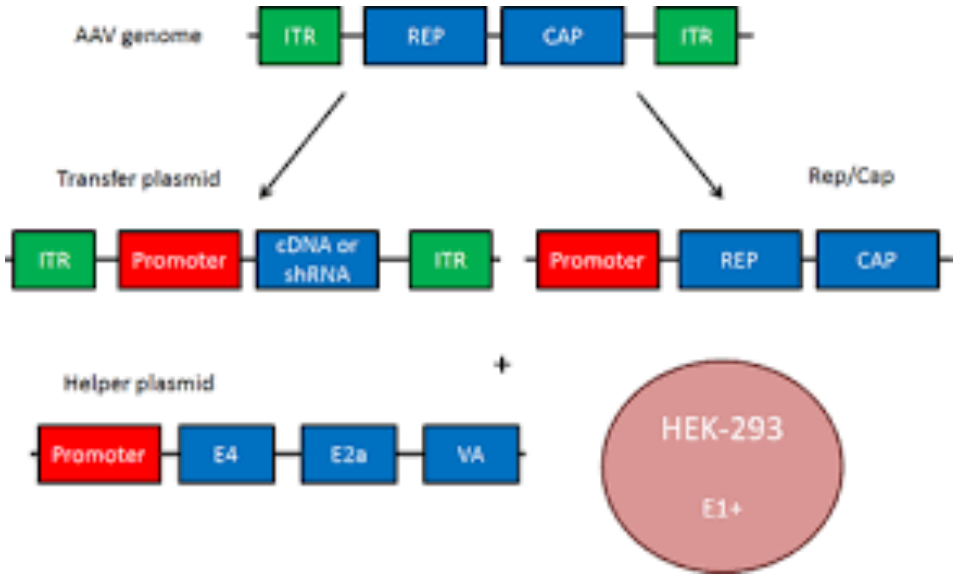
Stable expression

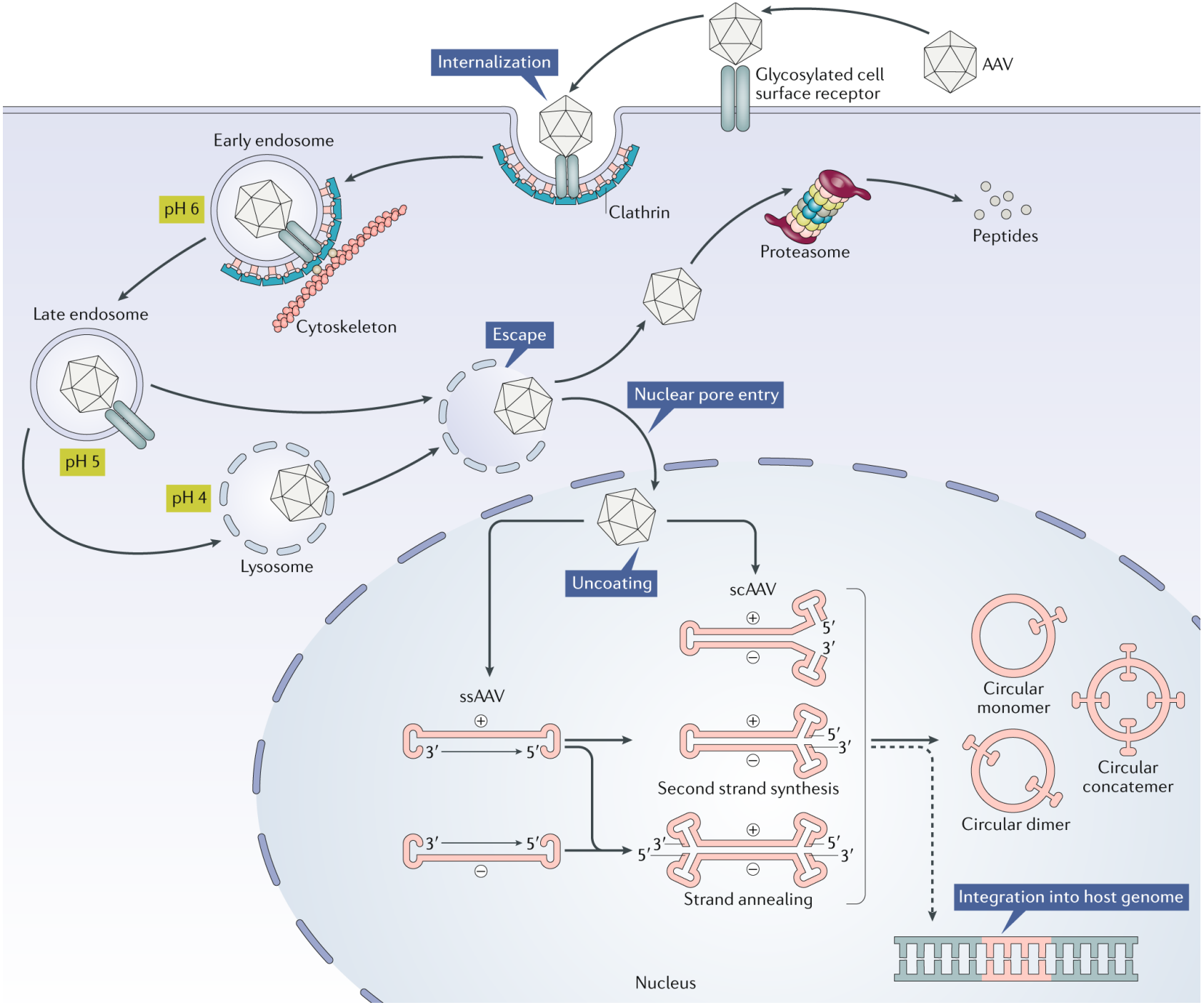
Cons:

Limited size of the host genes

The production requires a lot of work


HEK-293
cells
Helper
Ad Helper
Vector
Transfer
GOI
pAAV-ITR Vector
with GOI/shRNA
Rep/Cap
AAV
Rep/Cap Vector
Transfection
REP
CAP
Rep 78, 68, 52 & 40
cellular factors
Rep 78, 68
Replication
VP1
VP2
VP3
Assembly
Rep 52, 40
DNA packaging
AAV Viral Packaging
AAV
Particles
Gradient
Purification
(for all serotypes)
Desalting
and Titering
Purified
AAV Stock


AAV genome
ITR
REP
CAP
ITR
Transfer plasmid
Rep/Cap
ITR
Promoter
cDNA or
shRNA
ITR
Promoter
REP
CAP
Helper plasmid
+
Promoter
E4
E2a
VA
HEK-293
E1+

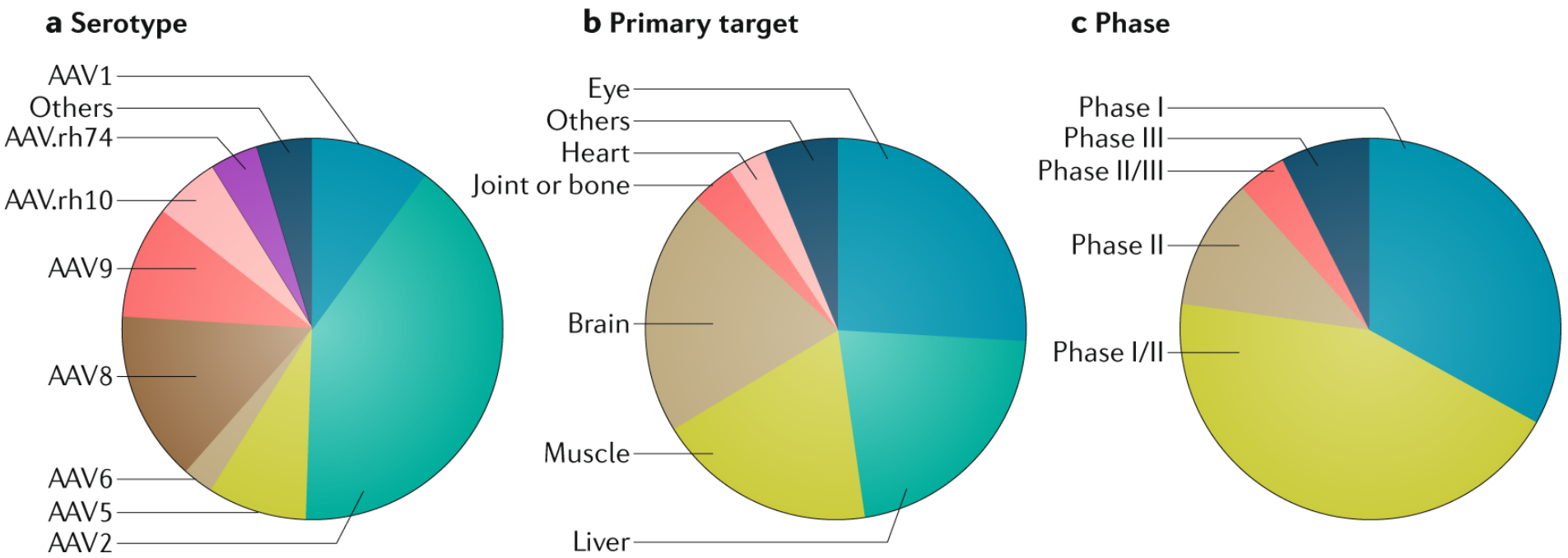

Internalization
Glycosylated cell surface receptor
AAV
Early endosome
pH 6
Clathrin
Proteasome
Peptides
Late endosome
Cytoskeleton
Escape
Nuclear pore entry
pH 5
pH 4
Lysosome
Uncoating
scAAV
ssAAV
Circular monomer
Circular concatemer
Second strand synthesis
Circular dimer
Strand annealing
Integration into host genome
Nucleus

AAV serotypes and their tropism

TARGET TISSUE	AAV Serotype
Skeletal muscle	AAV1***, AAV6**, AAV7*, AAV8***, AAV9***
Heart	AAV1***, AAV3*, AAV4*, AAV6**, AAV8*, AAV9**
Lung	AAV1*, AAV4**, AAV5**, AAV6**, AAV9***
Liver	AAV1*, AAV3***, AAV4*, AAV5**, AAV6*, AAV8**
T Cells	AAV6*
CNS	AAV1*, AAV2**, AAV5**, AAV6*, AAV7*, AAV8**, AAV9**
Hematopoietic stem cells	AAV2*, AAV3*, AAV6*
Embryonic stem cells, iPSC	AAV6*
Kidney	AAV2*, AAV4**, AAV5*
Pancreas	AAV1, AAV6*

Color Key: * mice / * large animals: dogs, pigs, sheep, rabbits, cats / * human (tissue or clinical trial) / AAV serotypes specificity verified by SIRION

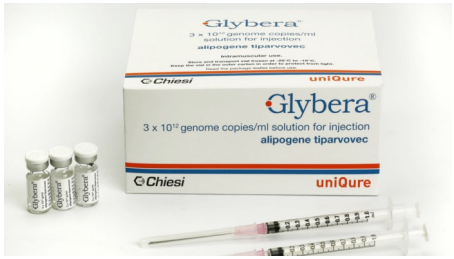
AAV and Gene Therapy



Wang D et al., Nature reviews drug discovery, 2019

FDA approved AAV in Gene Therapy

Name	Company	Disease Treatment	Approval Date	Serotype
Luxturna®	Spark Therapeutics, Inc.	RPE65-mutation-associated retinal dystrophy	2017	AAV2
Zolgensma®	Novartis Gene Therapies, Inc.	certain types of spinal muscular atrophy (**SMA**)	2019	AAV9
Hemgenix®	CSL Behring LLC	certain kinds of Hemophilia B	2022	AAV5
Elevidys	Serepta Therapeutics	Duchenne muscular dystrophy	2023	AAVrh74
Roctavian™	BioMarin	Hemophilia A	2023	AAV5



AAV1-LPL

LPLD: Glybera (2012)

- In 2012, the European Committee approved alipogene tiparvovec (**Glybera**): an **AAV1 expressing the LPL gene**
- Administered in a single session, through a **series of injections in the leg muscles - up to 60**. The **treatment is supposed to last at least ten years**.
- Glybera was **the first gene therapy to receive marketing authorization in Europe**;
- It earned the name "million-dollar drug", causing its manufacturer, uniQure, to remove the drug after two years on the European market and only a single patient treated.
- As of 2018, only 31 people worldwide have received Glybera but uniQure has no plans to sell the drug in the United States or Canada

OTC Deficiency:

DTX301

AAV8-ODC

Simon Smith

30/01/2019: First patient benefits from gene therapy trial with AAV-ODC vector: DTX301 (By Ultragenyx)

A patient at the Queen Elizabeth Hospital Birmingham (QEHB) was the first person in the world to take part in a pioneering gene therapy trial for Ornithine Transcarbamylase (OTC) deficiency, a rare disease that causes toxic levels of ammonia to build up in the blood.

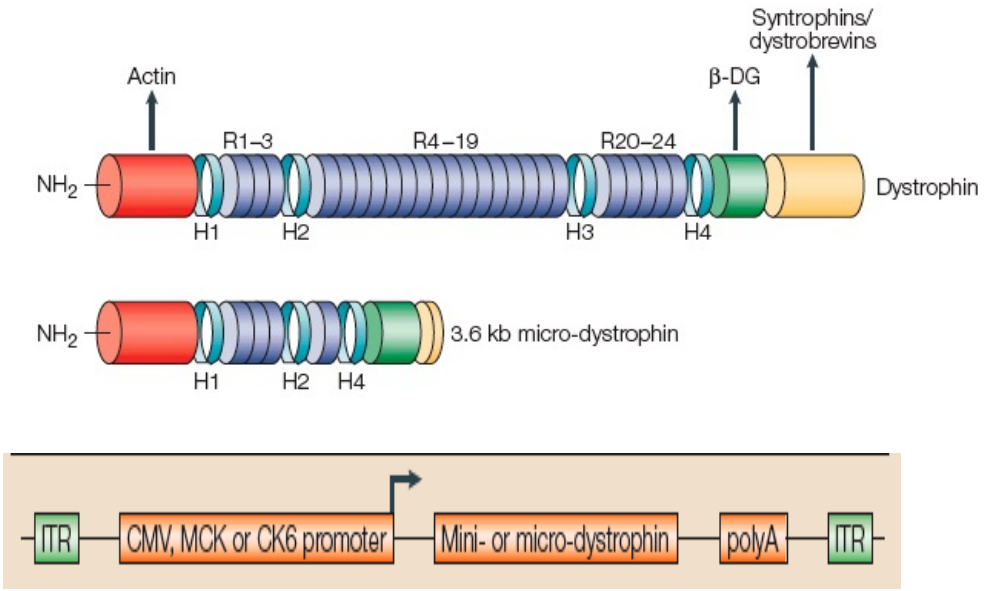
"The clinical trial Simon has taken part in uses a **one-time intravenous infusion gene transfer to deliver a working copy of the OTC gene to the liver**, offering a potential new way to treat the disease that directly targets the genetic defect, rather than just treating the symptoms. If successful – as seems to be the case for Simon – the liver cells will produce the OTC protein that is needed for a normal life."

Update Apr 22, 2021: **Phase 3 clinical** trial has been announced, based on the positive results from phase 1/2. **50 patients** will be enrolled, starting from the second half of 2021.

DMD: Elevidys

Delandistrogene moxeparvovec

- **Approved by FDA and EMA in 2023**
- **AAVrh74** expressing micro-dystrophin, a shortened but functional protein (138 kDa)
- Treatment of children aged four through five years of age
- **Single IV infusion** of 1-2 hrs



Lentivirus

- **RNA** viruses.

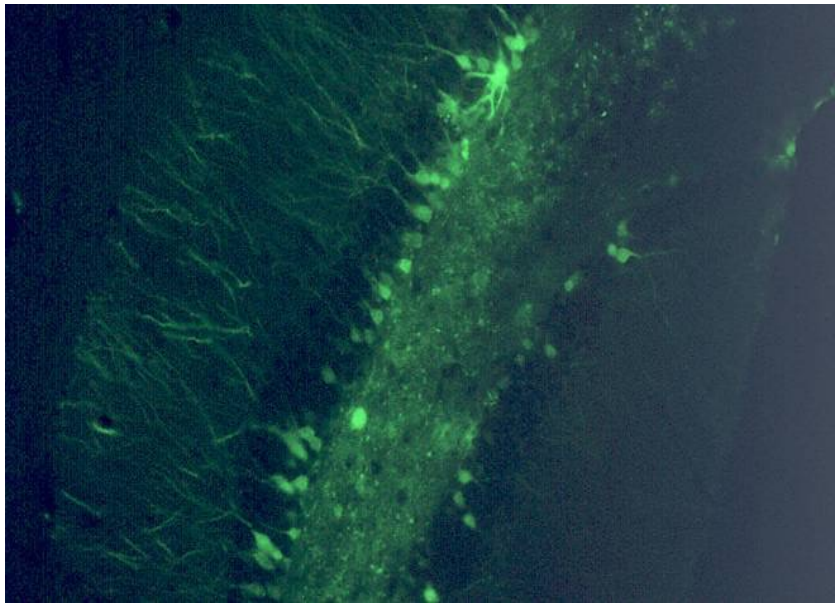
- May **infect non dividing cells** (e.g. heart and brain).

- Random **Integration in the genome** (insertional mutagenesis)

- Very **little immunological ractions**

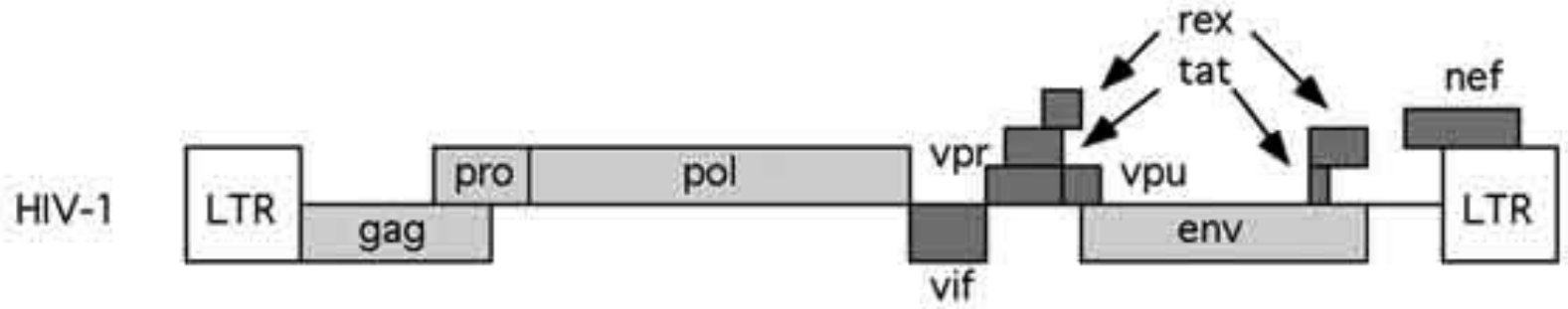
- **Elevated transduction efficiency**.

- Excellent perspective for in vivo GT.

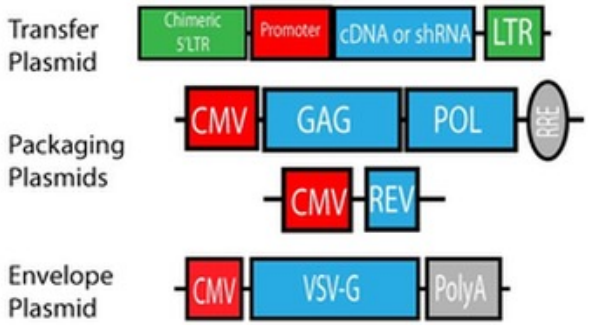


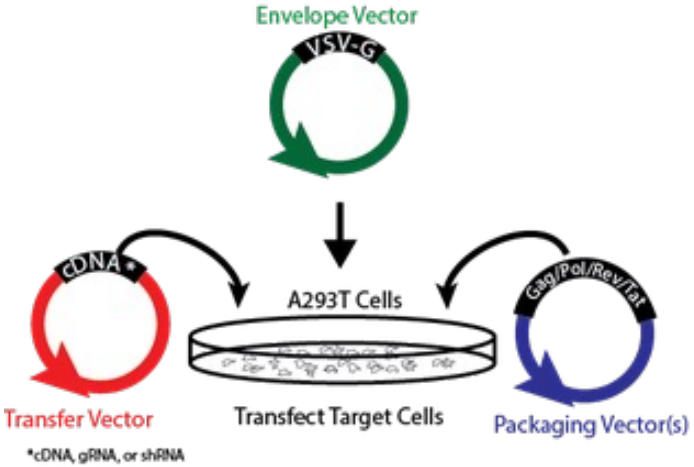
Lentiviral genome (HIV)

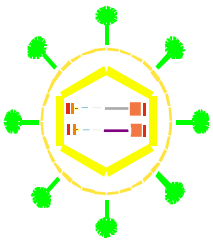
- Genes:
  - **structural** *gag pol env*
  - **regulatory** *tat rev*
  - **accessory** *nef vpr vpu vif*



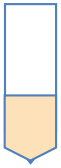
Lentiviral production





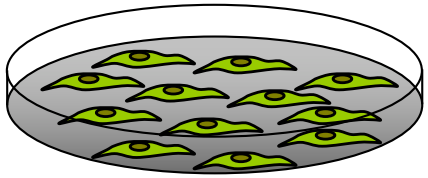


Harvest at 24 and 48 hrs



Concentration by ultracentrifugation

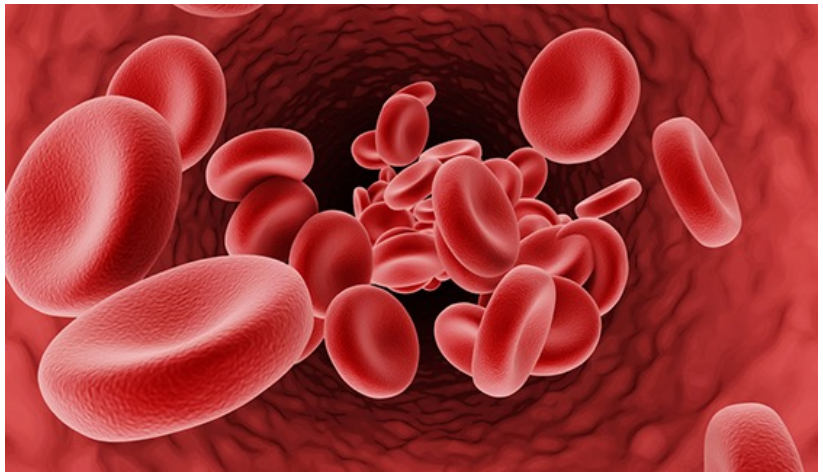
Titration



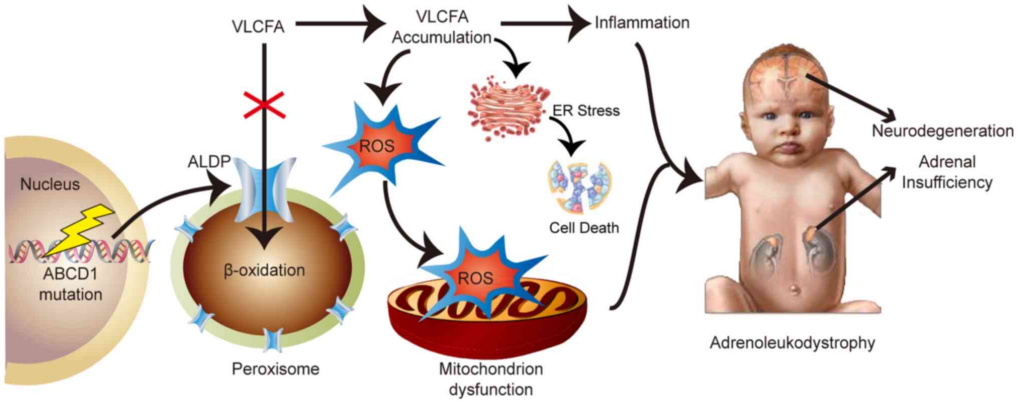
HeLa cells

Approved ex vivo GT with Lentiviruses

- **Zynteglo**
  - **Approved by FDA (2022) and EMA (2019)**
  - Treatment of people twelve years and older with **transfusion- dependent beta thalassemia (TDT)**
  - autologous **CD34+ hematopoietic stem cells** and progenitor cells transduced with the BB305 **lentiviral vector expressing the β-globin ($\beta^{A\text{-}T87Q}$) gene**



- **Skysona** (Elvandogene)
  - **Approved by FDA (2022) and EMA (2021)**
  - Treatment of **cerebral adrenoleukodystrophy (CALD, X-Linked)** in boys aged 4 to 17
  - **HSC** transduced with a **lentivirus expressing the ABCD1 gene**



Very long chain fatty acids accumulation

Table 1. Current FDA-approved gene therapies

Name	Vehicle	Target Gene	Disease	FDA-Approval	Cost/Patient (USD)	2022 Sales (USD)	Projected 2028 Sales (USD)
Luxturna	**AAV2**	**RPE65**	**Leber Congenital Amaurosis**	**May 2017**	**$850,000 ($425,000 per eye; one-time)**	**$125M**	**$126M**
Zolgensma	AAV9	SMN1	Spinal Muscular Atrophy (SMA)	May 2019	$2.1M (one-time)	$1.37M	$1.985M
Zynteglo	**LV**	**HBB**	**Beta Thalassemia**	**August 2022**	**$2.8M (one-time)**	**$3M**	**$162M**
Skysona	LV	ABCD1	Cerebral Adrenoleuko-dystrophy	September 2022	$3M (one-time)	$1M	$41M
Hemgenix	**AAV5**	**FIX**	**Hemophilia B**	**November 2022**	**$3.5M (one-time)**	**-**	**$67M**
Vyjuvek	HSV-1	COL7A1	Dystrophic Epidermolysi-s Bullosa	May 2023	$631,000 per year ($24,250 per vial)	-	$636M
Elevidys	**AAVrh74**	**DMD**	**Duchenne Muscular Dystrophy (DMD)**	**June 2023**	**$3.2M (one-time)**	**-**	**$2.731M**
Roctavian	AAV5	FVIII	Hemophilia A	June 2023	$2.9M (one-time)	-	$1.430M

CASGEVY:

THE FIRST CRISPR/Cas9-BASED THERAPY

Approved in UK **Nov 16th 2023**
For the treatment of **Sickle cell Anemia**