Biologia Molecolare 3.0

Cos'è l'ingegneria genetica?

Consiste nell'utilizzo di tecniche sperimentali per produrre molecole di DNA che contengono nuovi geni o nuove combinazioni di geni

Le tecniche dell'ingegneria genetica coinvolgono spesso l'isolamento, la manipolazione e la reintroduzione del DNA all'interno di cellule eterologhe o, più nello specifico, di organismi modello

Cos'è l'ingegneria genetica?

Consiste nell'utilizzo di tecniche sperimentali per produrre molecole di DNA che contengono nuovi geni o nuove combinazioni di geni

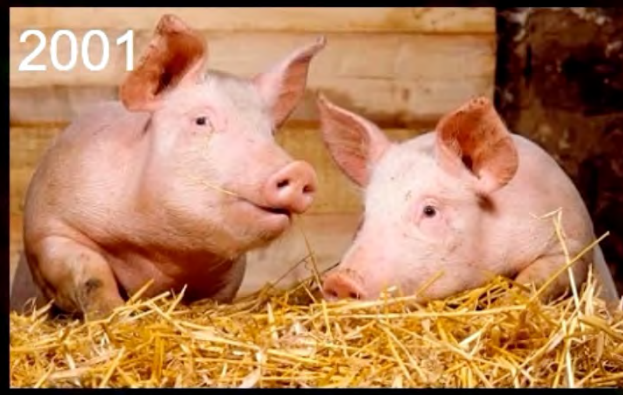
Perchè?

- Per la ricerca pura molto utili per comprendere a fondo la funzione di una determinata proteina
- Per la ricerca applicata, il fine ultimo è quello di conferire a determinati organismi caratteristiche importanti per svolgere determinati scopi.

Agricolo: ad es. cereali resistenti agli erbicidi)

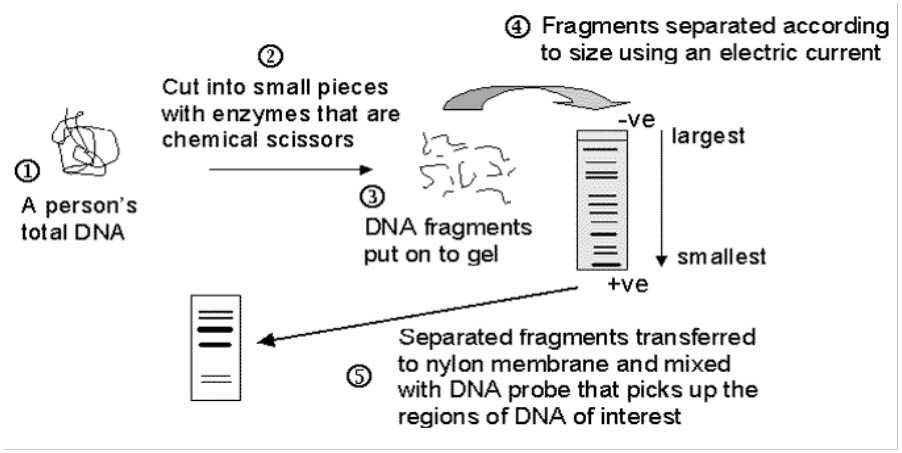
Biomedico: produzione di farmaci ricombinanti (ad es. la produzione di insulina attraverso batteri)

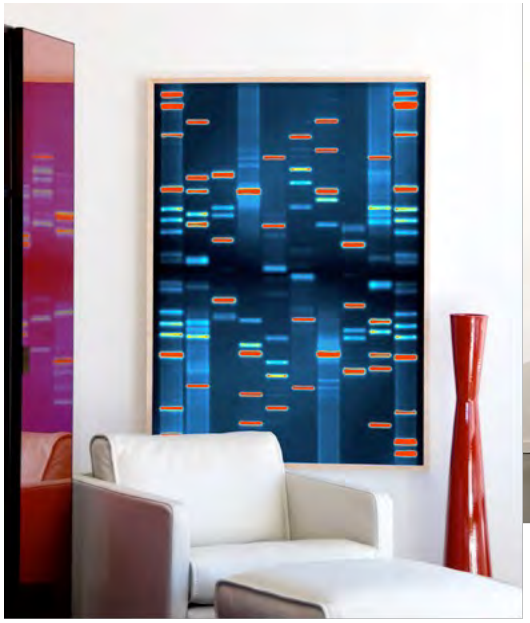
Genetically modified animals

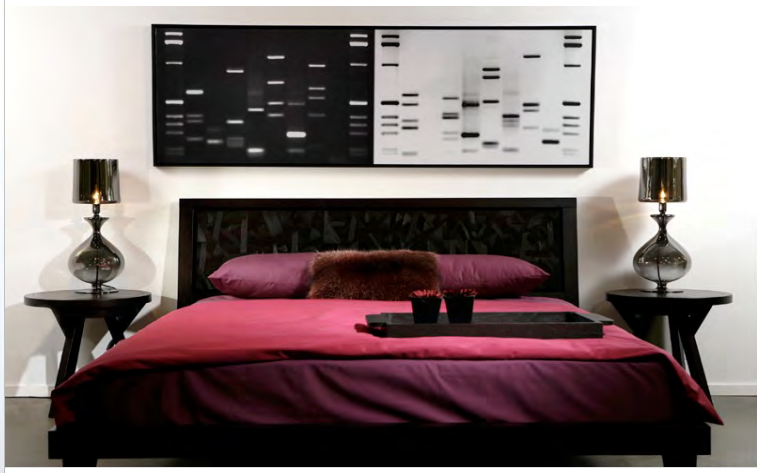


Ritratto del proprio DNA

DNA genomico estratto dalle cellule della mucosa della bocca
Digestione del DNA
Corsa elettroforetica
Si ottiene una combinazione di bande unica per ogni individuo





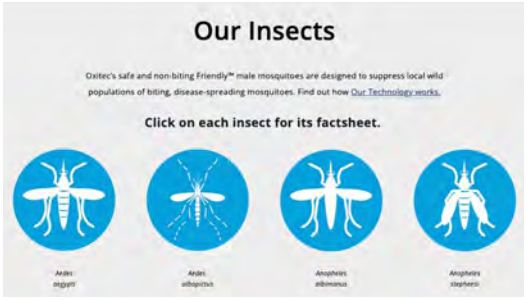


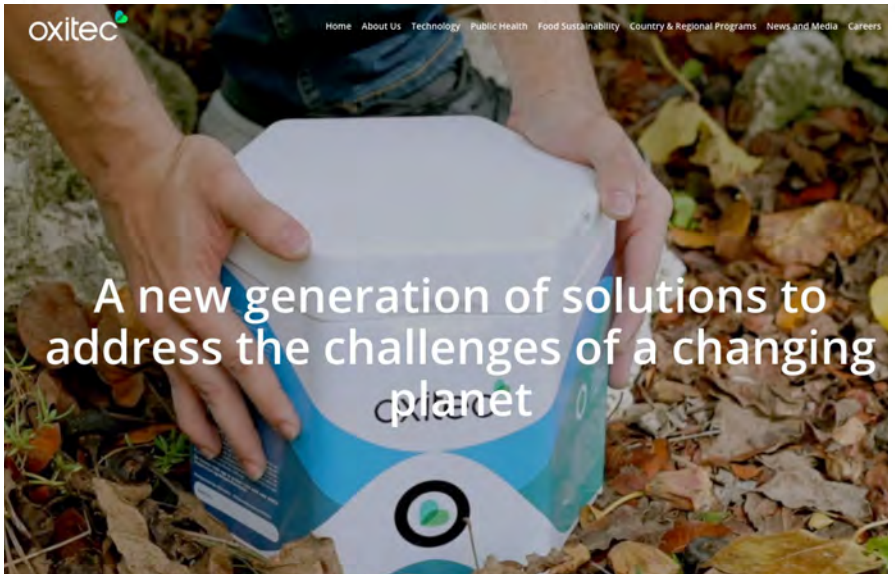
SCIENCE & NATURE

Can Mosquitoes Fight Malaria?

Scientists can build a mosquito that resists infection, but getting the insects to pass along the gene is a harder task

By Eric Jaffe





Genetic elimination of dengue vector mosquitoes

Megan R. Wise de Valdez[a,1], Derric Nimmo[b], John Betz[a], Hong-Fei Gong[b], Anthony A. James[c,2], Luke Alphey[b,d,2], and William C. Black IV[a]

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Contributed by Anthony A. James, January 10, 2011 (sent for review September 29, 2010)

Oxitec released 3.3 milion sterile male transgenic *Aedes aegypti* mosquitoes in a field trial aimed at reducing wild mosquito populations to control dengue.

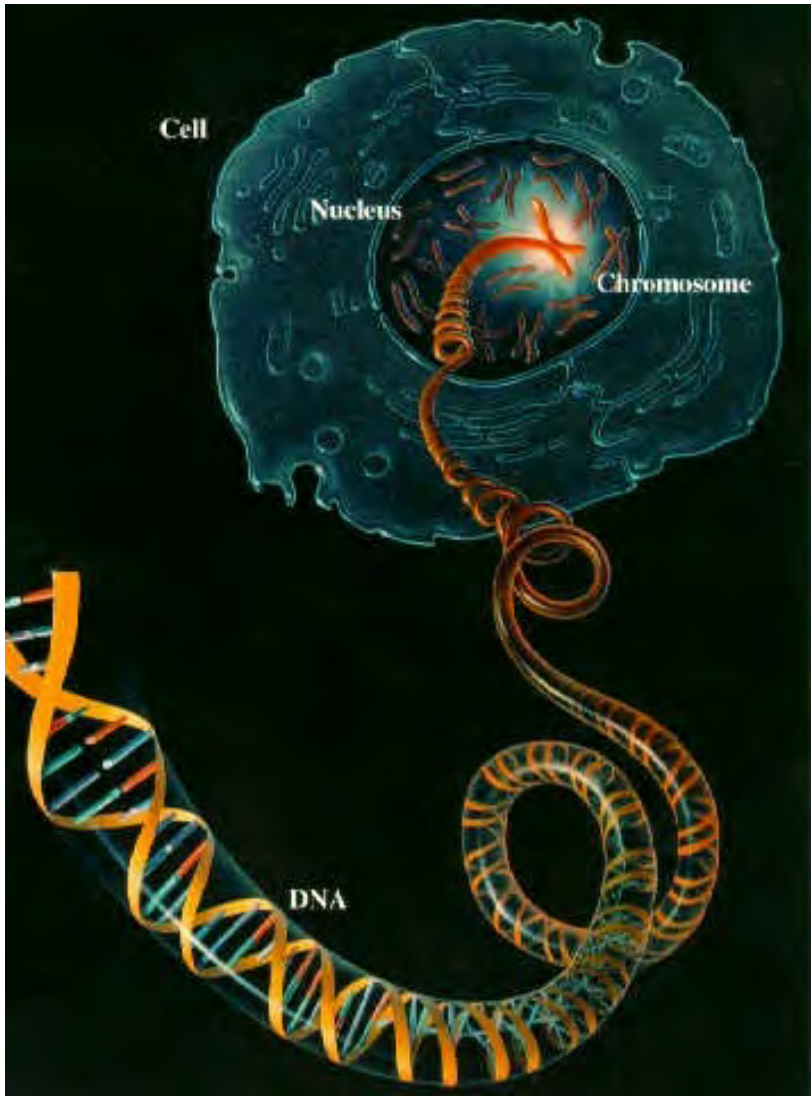
FDA/EMA approved gene therapy products (18)

Product	Company	Year	Therapeutic gene	Disease	Vector	Prevalence / Incidence	Price (USD)
Glybera	UniQure	2012	Lipoprotein lipase	Lipoprotein lipase deficiency (LPLD)	AAV1	1:1,000,000	1M
Strimvelis	GlaxoSmithKline	2016	Adenosine deaminase	ADA-SCID	GRV	1:100,000	665K
Luxturna	Spark Therapeutics	2017	RPE65	Leber's congenital amaurosis (LCA)	AAV2	<1:100,000	435K/eye
Zynteglo	Bluebird Bio	2022	Beta globin	Beta thalassaemia	LV	1:100,000	2.8M
Zolgensma	Novartis/Avexis	2019	SMN1	Spinal muscular atrophy (SMA)	AAV9	1-2:100,000	2.1M
Hemgenix	Behring	2022	Factor IX	Haemophilia B	AAV5	3.7:100,000	3.5M
Skysona	Bluebird Bio	2022	ABCD1	X-linked adrenoleukodystrophy	LV	5:100,000	3M
Libmeldy	Orchard Therapeutics	2020E	Arylsulfatase A (ARSA)	Metachromatic leukodystrophy (MLD)	LV	2.5-10:100,000	3.9M
Upstaza	PTC Therapeutics	2022E	AADC	Aromatic L-amino acid decarboxylase deficiency	AAV2	1.1:100,000	
Roctavian	BioMarin	2022E	Factor VIII-SQ	Haemophilia A	AAV5	12:100,000	2.5M
Yescarta	Kite Pharma/Gilead	2017	CAR-T (CD-19)	Diffuse Large B-cell NHL	GRV	4:100,000 per year	373K
Kymriah	Novartis	2017	CAR-T (CD-19)	B-cell lymphoma	LV	1.7:100,000	475K
Breyanzi	Bristol Myers Squibb	2022	CAR-T (CD19)	B-cell lymphoma	LV	1,7:100,000/year	471K
Abecma	Bristol Myers Squibb	2021	CAR-T (BCMA)	Multiple myeloma	LV	1-2:100,000/year	480K
Carvykti	Janssen	2023	CAR-T (BCMA)	Multiple myeloma	LV	1-2:100,000/year	490K
Tecartus	Kite Pharma	2020	CAR-T (CD-19)	Mantle cell lymphoma	GRV	4-8/1M/year	373K
Adstiladrin	Ferring	2023	Interferon alfa-2b	Bladder cancer	Ad	32:100,000/year	~200K
Imlygic	Amgen/BioVex	2023	Oncolytic herpesvirus	Melanoma	Herpesvirus	25:100,000/year	65K

https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/approved-cellular-and-gene-therapy-products
https://www.genetherapynet.com/gene-therapy-products-on-the-market.html
https://www.pei.de/EN/medicinal-products/atmp/gene-therapy-medicinal-products/gene-therapy-node.html

TERAPIA GENICA

- Introduzione di un transgene in una cellula allo scopo di correggere un errore innato o per fornire una nuova funzione cellulare o per neutralizzare un prodotto espresso dalla cellula



Terapia genica somatica: quali I migliori candidati?

- Malattie monogeniche, con ereditarietà recessiva o legata all'X
- Morbilità o mortalità significative: tumori e malattie cardiovascolari
- Terapia attuale inadeguata o non disponibile
- La sede cellulare del difetto genetico deve essere facilmente accessibile

Malattie monogeniche candidate alla GT

Disease	Defect	Incidence	Target Cells
Severe combined immunodeficiency (SCID)	Adenosine deaminase (ADA) in 25% of SCID patients	Rare	Bone-marrow cells or T lymphocytes
Hemophilia A	Factor VII deficiency	1:10,000 males	Liver, muscle, fibroblasts or bone marrow cells
Hemophilia B	Factor IX deficiency	1:30,000 males	
Familial hypercholesterolemia	Deficiency of low-density lipoprotein (LDL) raeceptor	1:1 million	Liver
Cystic fibrosis	Faulty transport of salt in lung epithelium	1:3000 Caucasians	Airways in the lungs
Hemoglobinopathies thalassemias	(Structural) defects in the α or β globin gene	1:600 in certain ethnic groups	
Gaucher's disease	Defect in the enzyme glucocerebrosidase	1:450 in Ashkenazi Jews	Bone marrow cells, macrophages
α_1 antitrypsin deficiency inherited emphysema	Lack of α_1 antitrypsin	1:3500	Lung or liver cells
Duchenne muscular distrophy	Lack of dystrophin	1:3000 males	Muscle cells

Malattie ad alta mortalita' candidate alla GT

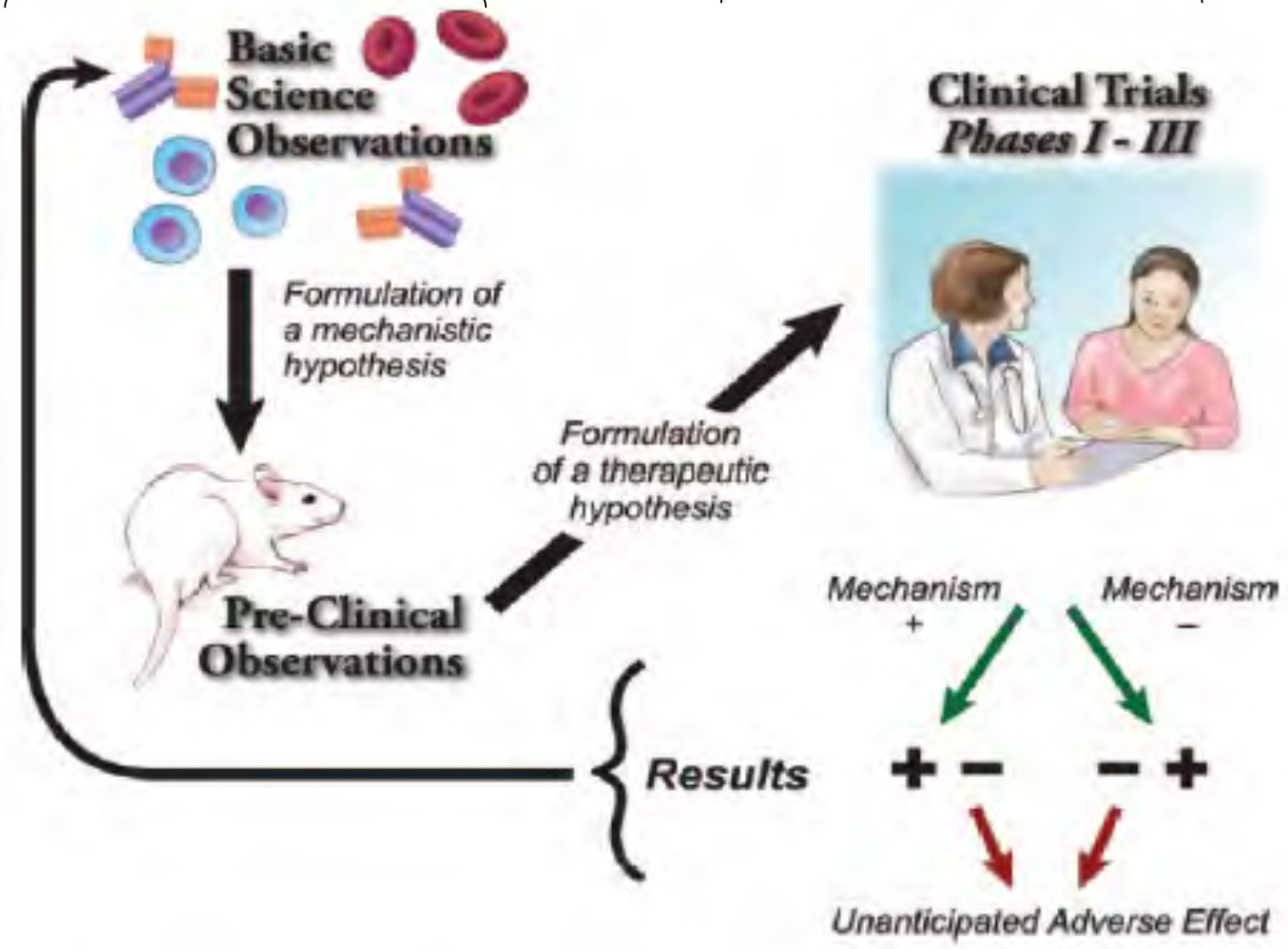
Some Acquired Diseases that are Candidates for Gene Therapy

Disease	Defect	Incidence	Target Cells
Cancer	Many causes, including genetic and environmental	1 million/year in USA	Variety of cancer cell types, in liver, brain, pancreas, breast, kidney
Neurological diseases	Parkinson's, Alzheimers's spinal-cord injury	1 million Parkinson's and 4 million Alzheimer's patients in the USA	Neurons, glial cells, Schwann cells
Cardiovascular	Restenosis, arteriosclerosis	13 million in USA	Arteries, vascular endothelia walls
Infectious diseases	AIDS, hepatitis B	Increasing numbers	T cells, liver, macrophages
Rheumatoid arthritis	Autoimmune inflammation of joints	Increasing numbers with aging population	

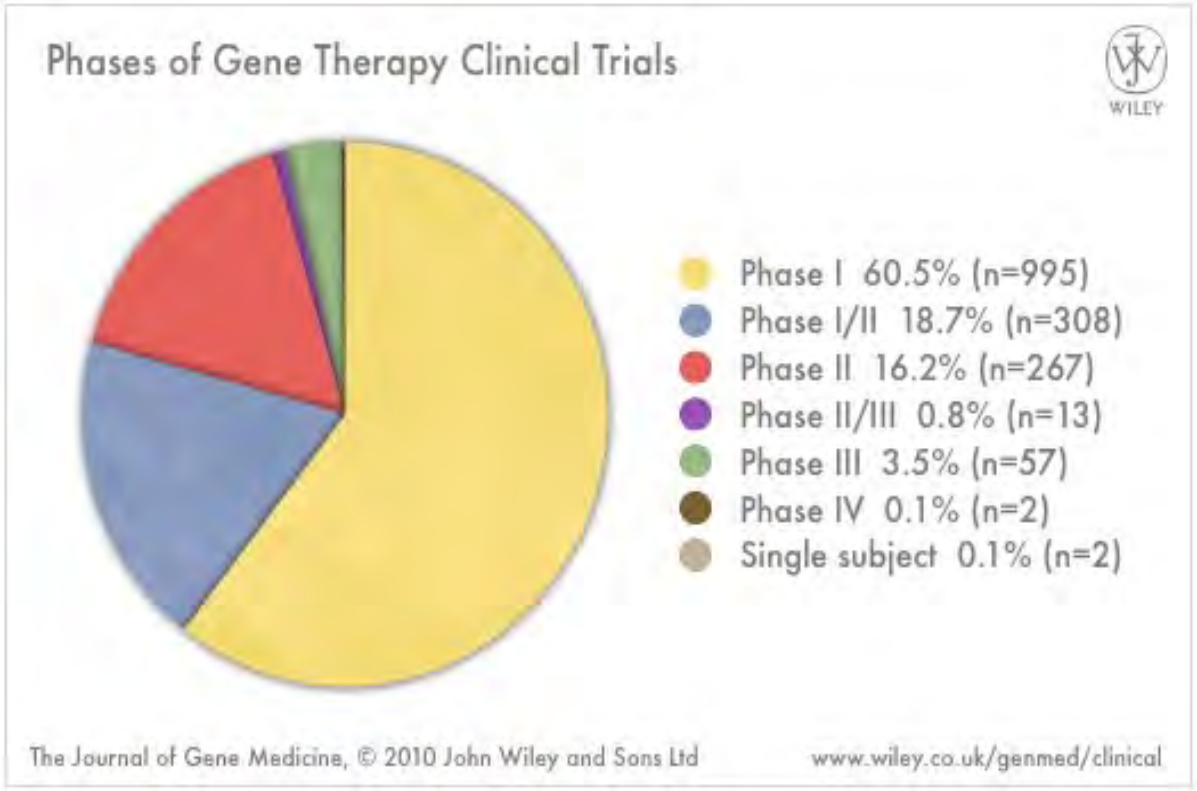
Sviluppo di una nuova terapia

Ricerca pre-clinica

Sperimentazione clinica (trial)



Trial clinici in GT



Una sperimentazione clinica (clinical trial) e' caratterizzata da diverse fasi:

- Fase I *(quanto farmaco può essere somministrato senza causare effetti avversi gravi?)*
- Fase II (*valutare come funziona il farmaco, e continuare la valutazione sulla sicurezza di fase I, su un gruppo più ampio di volontari e pazienti)*
- Fase III *(Quando un farmaco è considerato efficace e sicuro, viene somministrato a un numero alto di pazienti)*
- Fase IV *(Detta anche sorveglianza Post-Marketing per trovare ogni evento avverso raro od a lungo tempo, su una più grande popolazione di pazienti)*

Terapia genica: come?

ex vivo

Le cellule bersaglio sono prelevate dal paziente, modificate geneticamente in laboratorio e reintrodotte nello stesso individuo

✓ no problemi immunologici

efficienza delle metodiche di trasduzione in vitro

✓ solo alcune malattie (immunologiche, ematologiche, metaboliche)

in situ

il transgene viene rilasciato localmente nel sito di azione mediante iniezione i.m. o intratumorale o per inalazione ecc…

✓tumori localizzati; patol. dell'apparato respiratorio; tessuto cutaneo ecc…

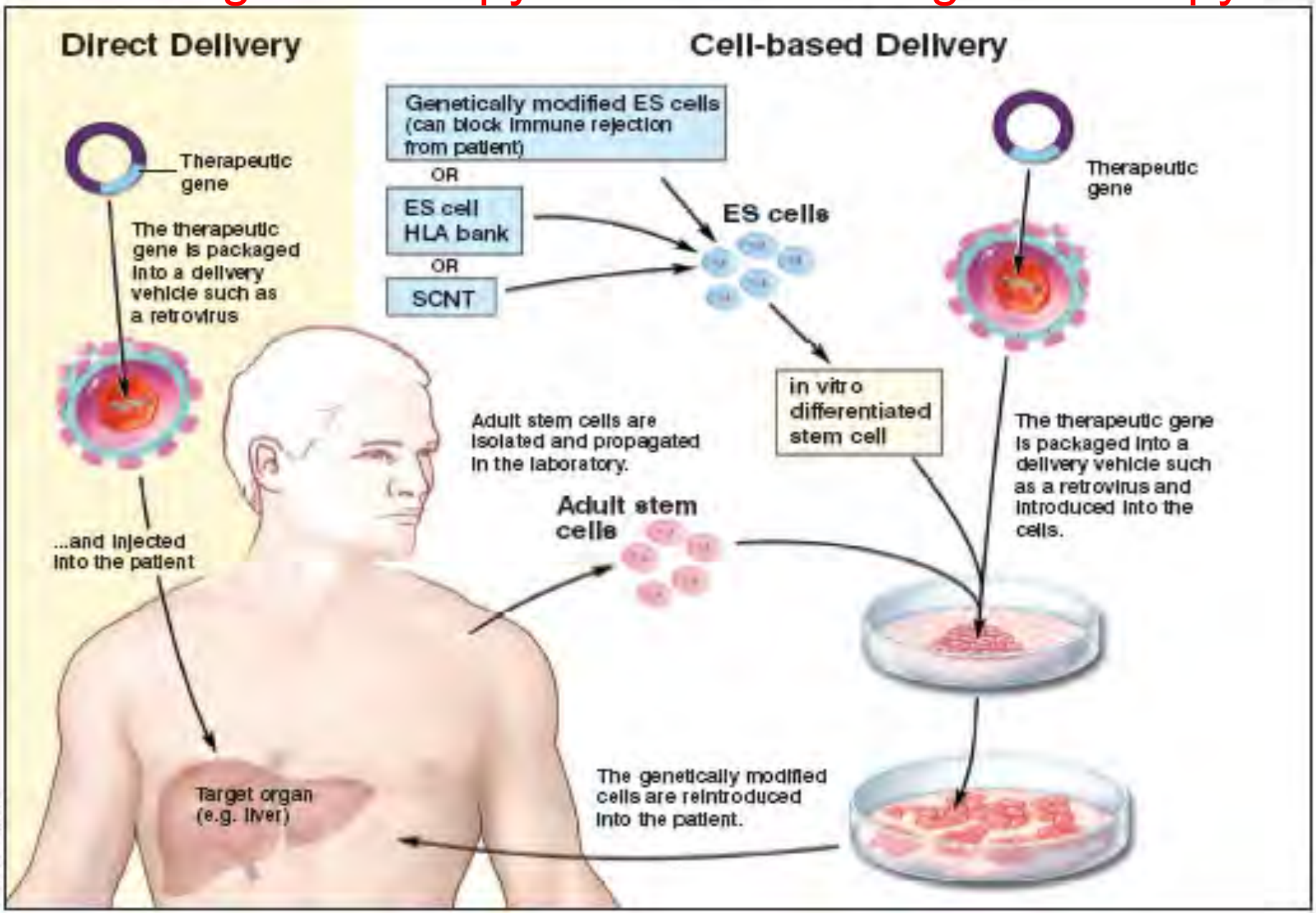
in vivo

il transgene viene somministrato per via sistemica e.v. nel corpo del paziente

- cellule e tessuti poco accessibili

✓scarsa efficienza di trasduzione, barriere

In vivo gene therapy ***Ex vivo*** gene therapy



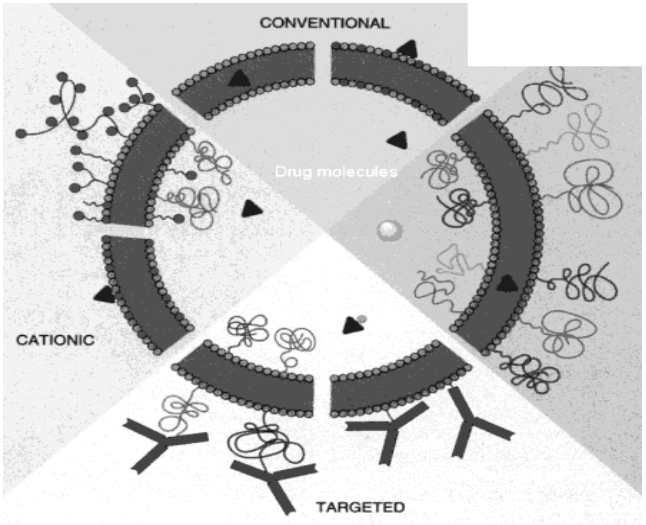
Tecniche di trasferimento genico

Lipofezione

Liposomi

Lipidi cationici

Complessi liposomi/proteine



Vettori virali

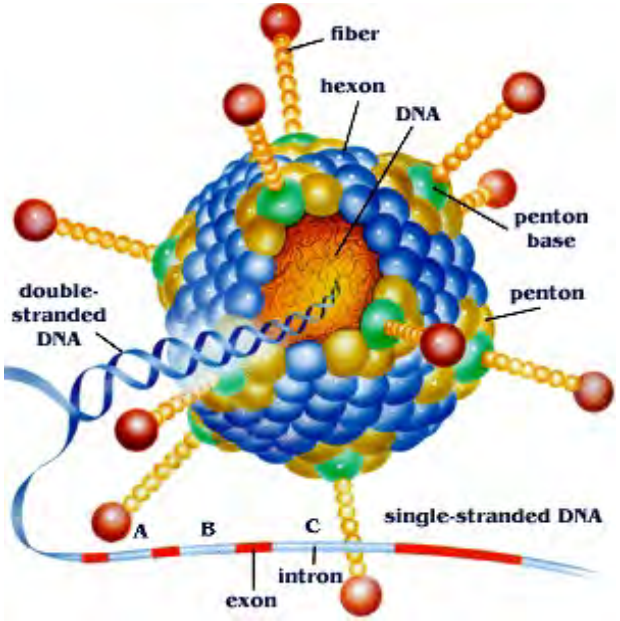
Retrovirus

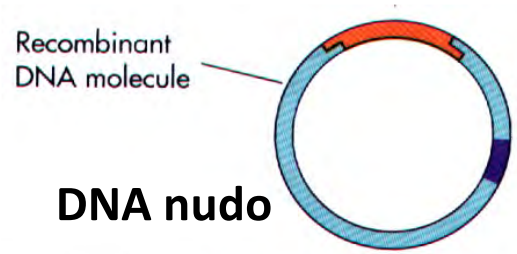
Adenoviruses

Virus adeno-associati (AAV)

Lentiviruses

Herpesviruses





Metodi fisici

Microiniezione

Elettroporazione

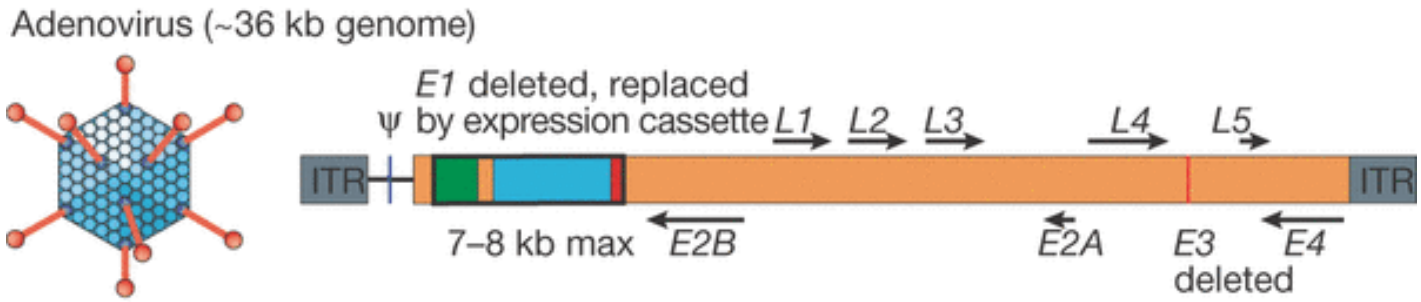
DNA complessato a nanoparticelle

Gene gun ("spara" nella cellula particelle microscopiche d'oro o tungsteno ricoperte da DNA).

Vettore Virale ideale

- ✓ di facile produzione e in elevate quantità
- ✓ esprimibile per un lungo periodo e regolabile
- ✓ sicuro, cioé inerte dal punto di vista immunologico
- ✓ selettivo per determinati tipi cellulari
- ✓ capace di trasportare geni piccoli e grandi
- ✓ capace di integrarsi in siti specifici del genoma
- ✓ capace di infettare sia cellule in divisione che quiescenti

Vettori virali



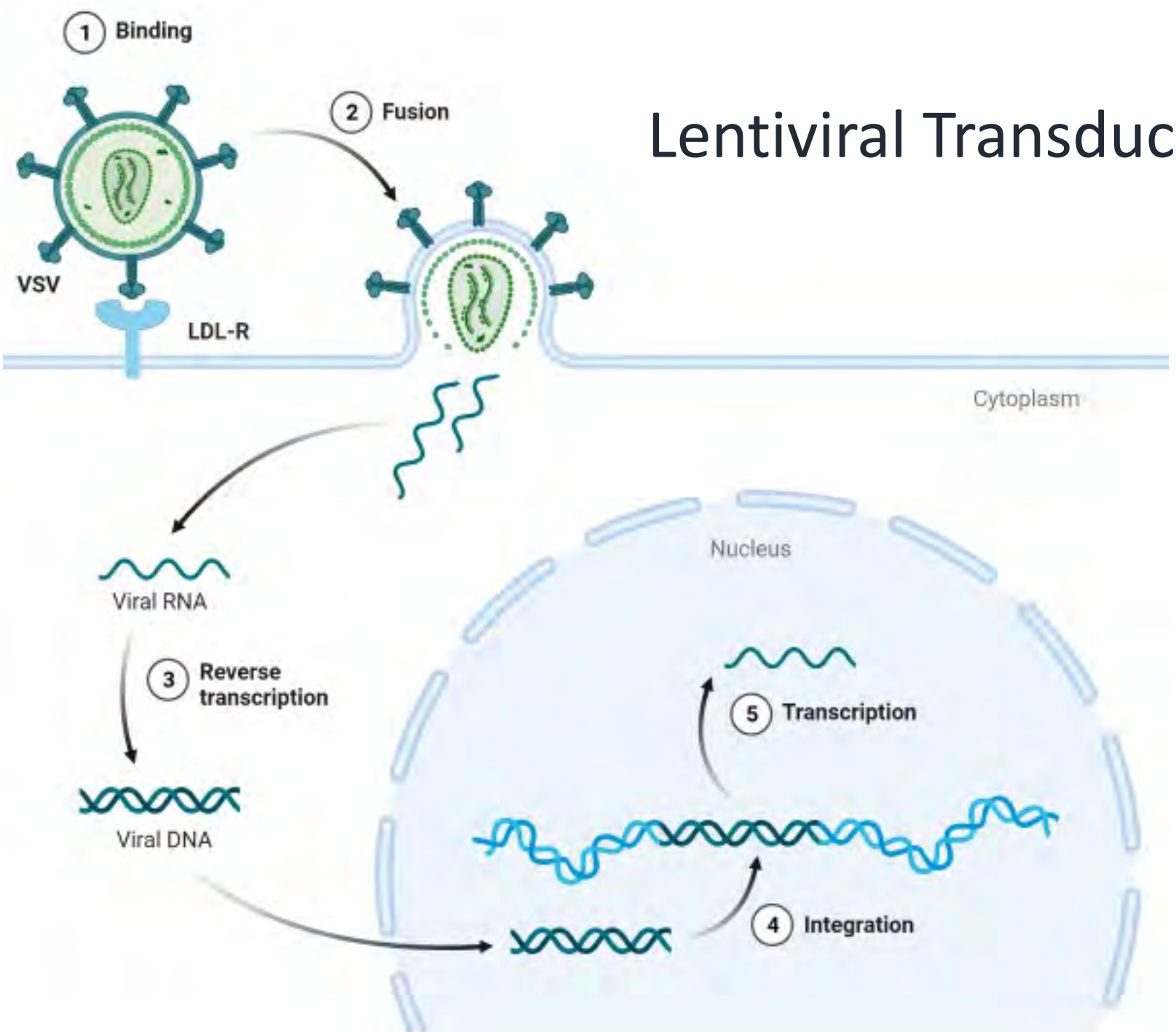
Vantaggi

- ✓ altamente efficienti nel trasferimento genico
- ✓ espressione a lungo-termine

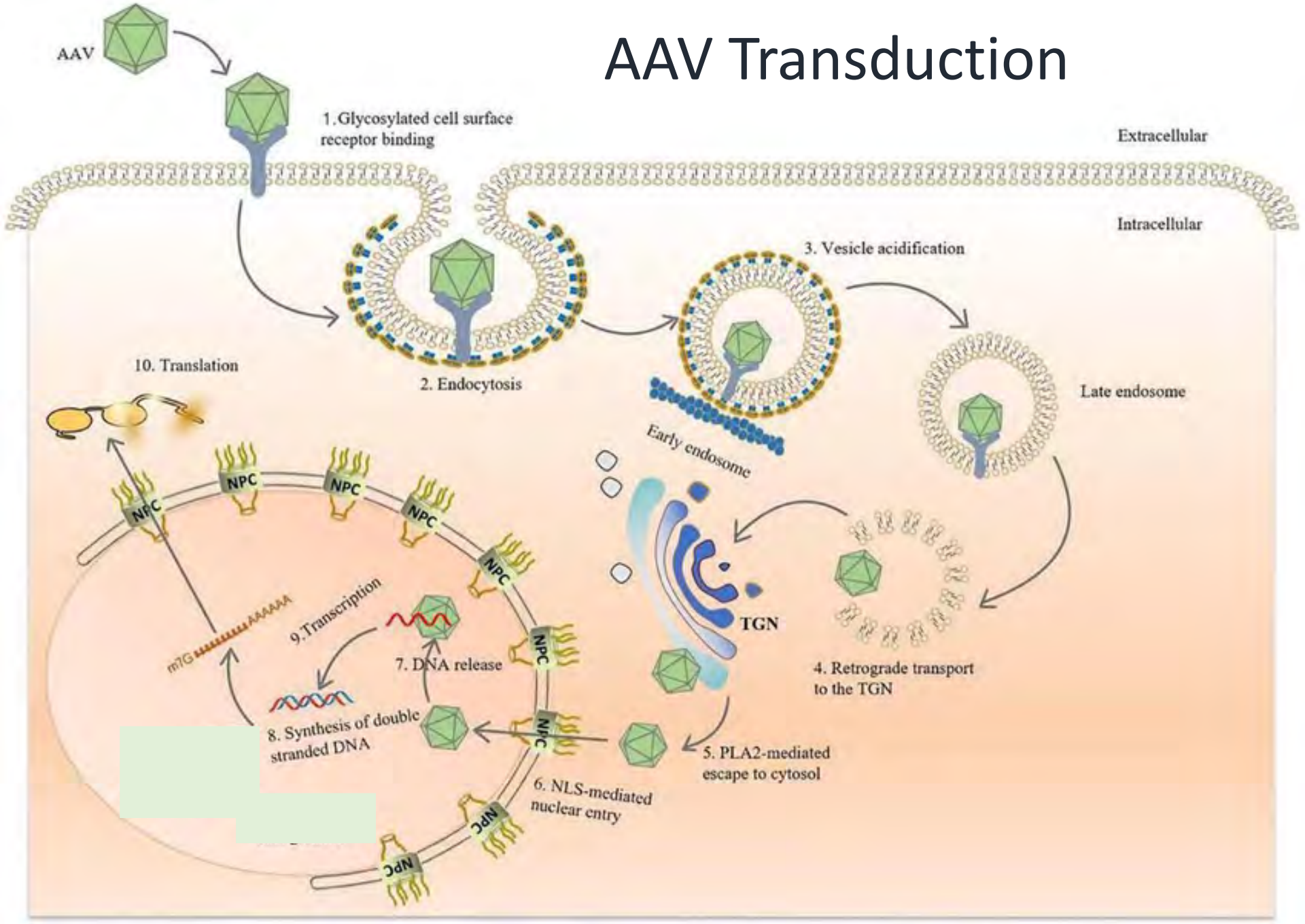
Svantaggi

- ✓ reazione immunitaria
- ✓ tossicità
- ✓ integrazione random/mutagenesi inserzionale*

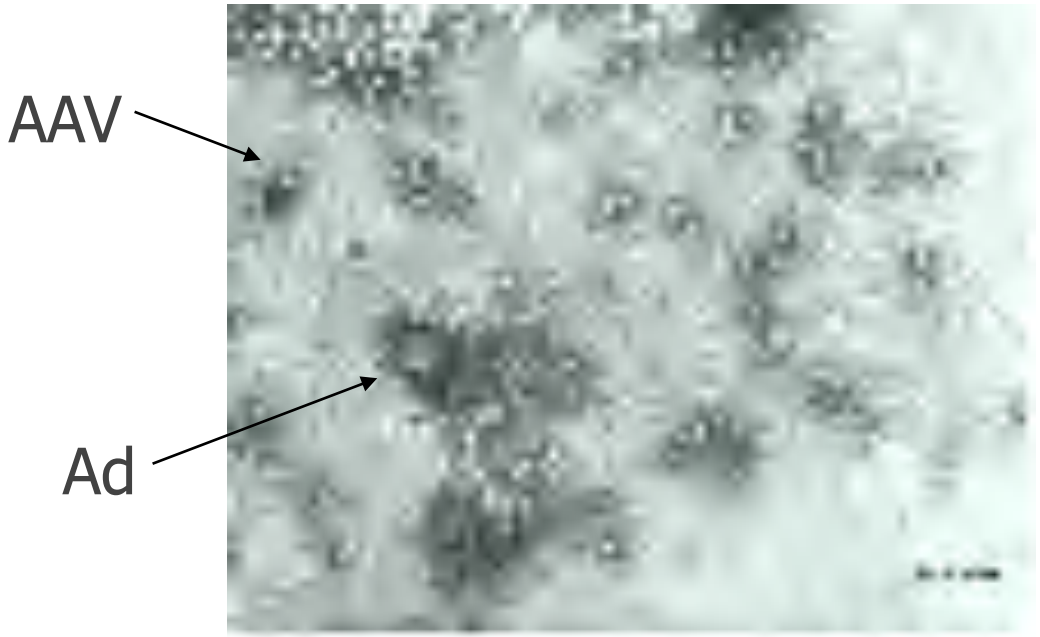
Lentiviral Transduction



AAV Transduction



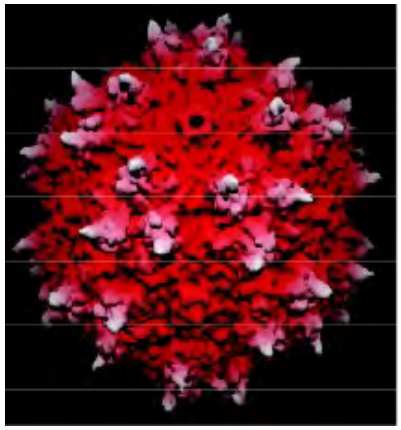
Adeno-associated virus (AAV)



Taxonomy
Family: Parvovirus
Subfamily: Parvoviridae
Genus: Dependovirus
Type: AAV 1-12

Morphology
Particles are icosahedral, non-enveloped,
18-26 nm diameter, 50% protein (VP1-3) 50% DNA.
Resistant to inactivation by solvents, pH and heat.

Genome
Linear, non-segmented, ssDNA ~5 kb.
AAVs package equal amounts of (+) and (-) strands.



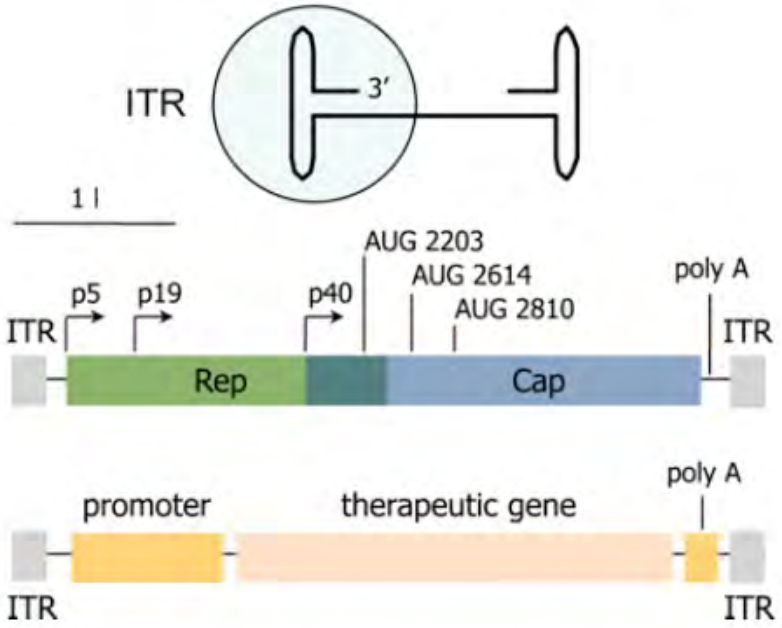
Xie et al. 2002

Gene transfer using Adeno-Associated Virus (AAV) vectors

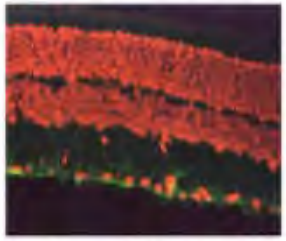
1. Based on a widely diffused, non pathogenic virus
2. Vectors retain less that 10% of the viral genome
3. Vectors do not express any viral protein (not inflammatory and not immunogenic); long term ensured in vivo
4. Expression of the therapeutic gene can be driven by any desirable promoter
5. High titer vector preparations are obtained by virion purification
6. Mixing of different rAAV preparations results in the simultaneous expression of gene combinations in vivo

Xie et al. 2002

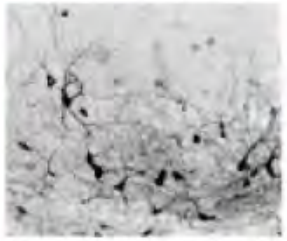
Family: Parvovirus
Genus: Dependovirus
Type: AAV 1-9
Size: 18-26 nm
Genome: ssDNA ~5 kb



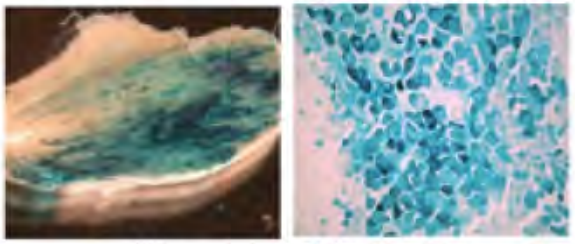
AAV vectors transduce with high efficiency post-mitotic tissues in vivo



Retina, AAV2-GFP



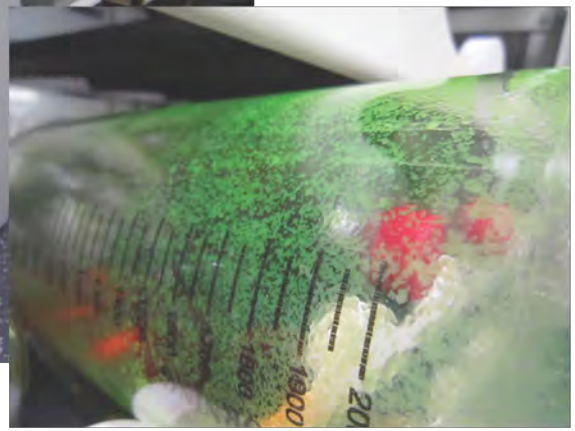
Brain, AAV2-bcl2

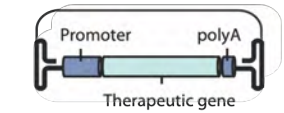


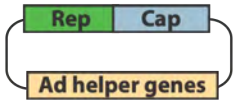
Muscle, AAV8-βgal

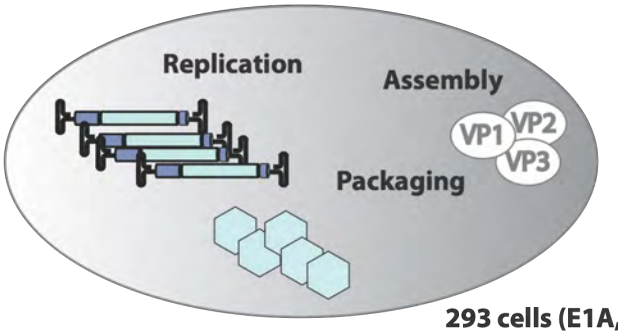
Heart, AAV9-βgal

AAV Vector Unit (AVU)
ICGEB Trieste

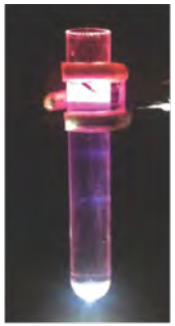


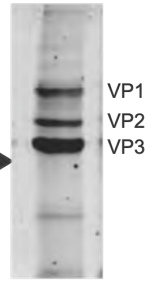




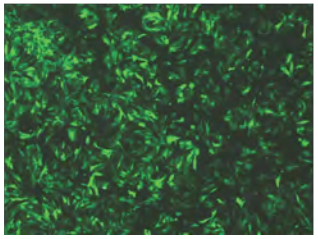


293 cells (E1A, E1B)

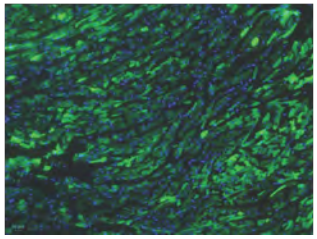




AAV6 neoCM in vitro



AAV9 heart tissue in vivo

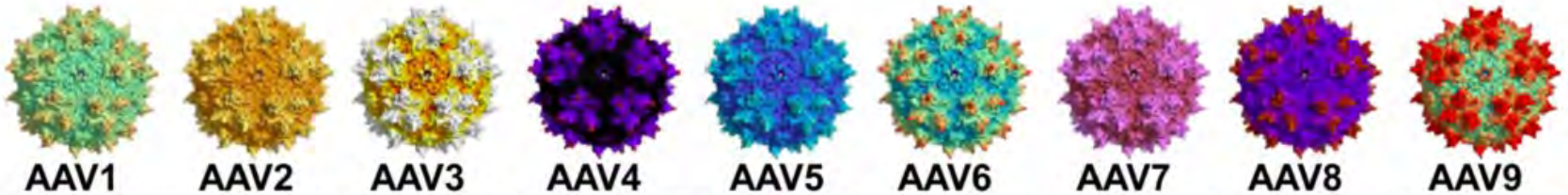


Production of avarage 250 batches of over 40 different vectors per year
Yield: **1x10^{13}-1x10^{14} viral particles/cell**
Physical Titer: **1x10^{12} to 1x10^{13} viral genomes/ml**

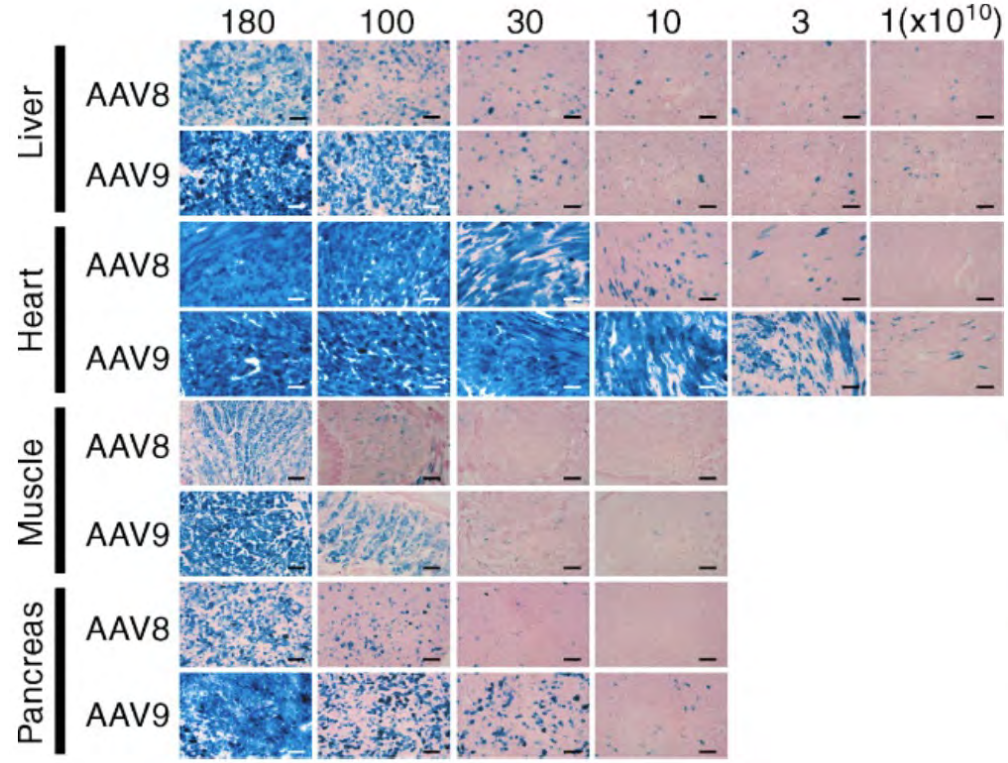
Recettori di alcuni Parvovirus

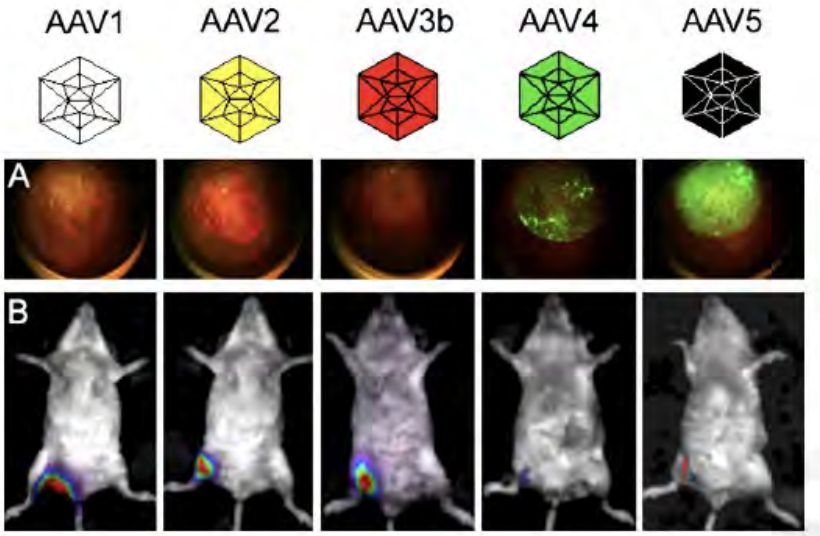
Parvovirus	Recettore
AAV1	Acido sialico (legami α2-3-N e α2-6-N)
AAV2	Proteoglicani contenenti eparan-solfati (HSPG) Corecettori: integrina αvβ5, FGFR1, HGF-R
AAV3	Proteoglicani contenenti eparan-solfati (HSPG)
AAV4	Acido sialico (legami α2-3-O)
AAV5	Acido sialico (legami α2-3-O e α2-3-N) Recettore del PDGF (PDGFR)
AAV6	Acido sialico (legami α2-3-N e α2-6-N)
AAV7	Non noto
AAV8	Recettore della laminina (LamR)
AAV9	Non noto (LamR?)
Parvovirus B19	Antigene P dei globuli rossi
CPV (parvovirus canino)	Recettore della trasferrina Acido sialico (acido N-glicolil-neuraminico, NeuGC)
FPV (parvovirus della panleucopenia felina)	Recettore della trasferrina

Biodistribution of AAV serotypes 1-9 in mice



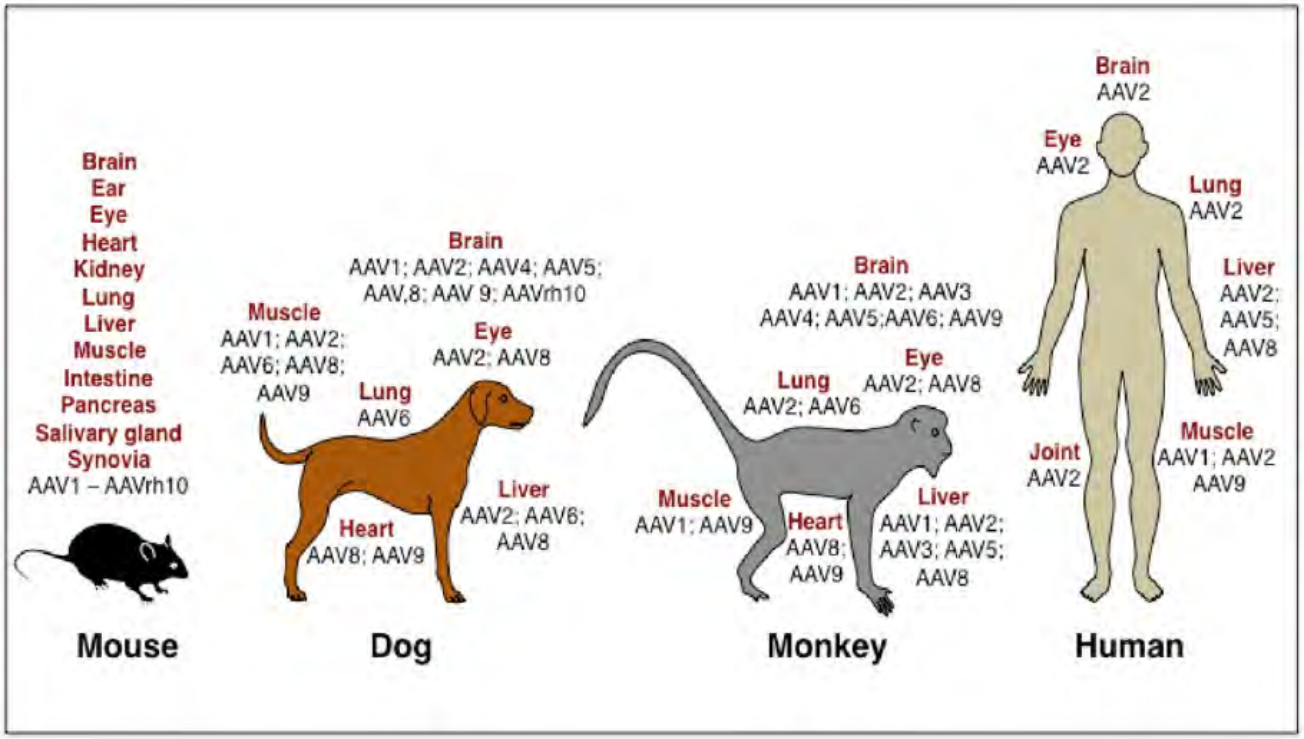
Mouse	AAV1	AAV2	AAV3	AAV4	AAV5	AAV6	AAV7	AAV8	AAV9
	Liver, heart, skeletal muscle	Liver, heart and muscle	Heart, Liver	Heart, lung, Liver	Liver	Liver, heart, skeletal muscle	Liver, skeletal muscle	Heart, Liver, brain, muscle	Liver, heart, brain, Lung, skeletal muscle



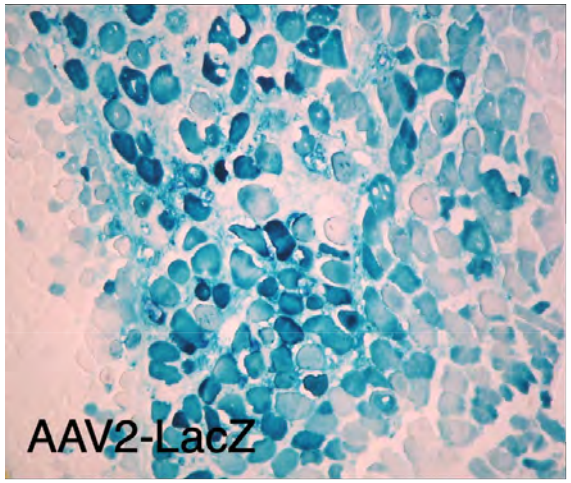


Criteria for the choice of the AAV serotype to use as a gene transfer vector

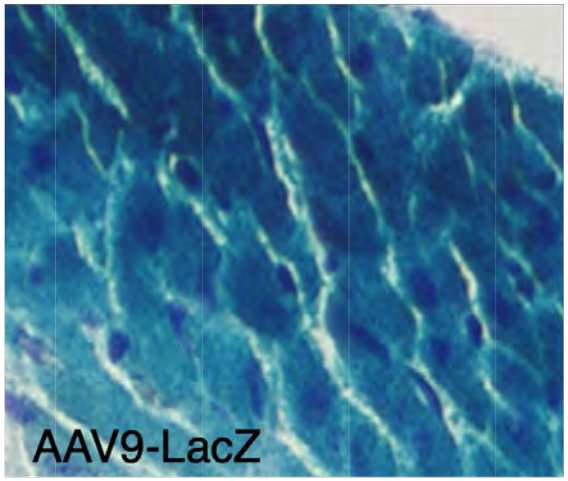
- ★ **Which cell/tissue types are being targeted**
- ★ The safety profile associated with the delivered gene
- ★ The choice of systemic versus local delivery
- ★ The use of tissue-specific or constitutively active promoters
- ★ Which animal species is the final target



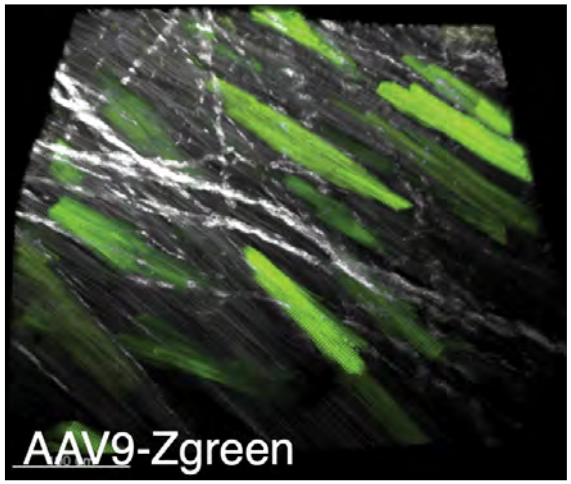
AAV efficiently transduces permissive tissues and promotes persistent transgene expression



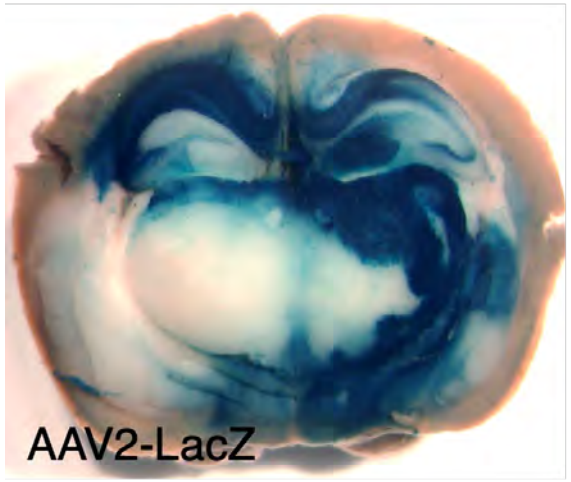
Skeletal muscle



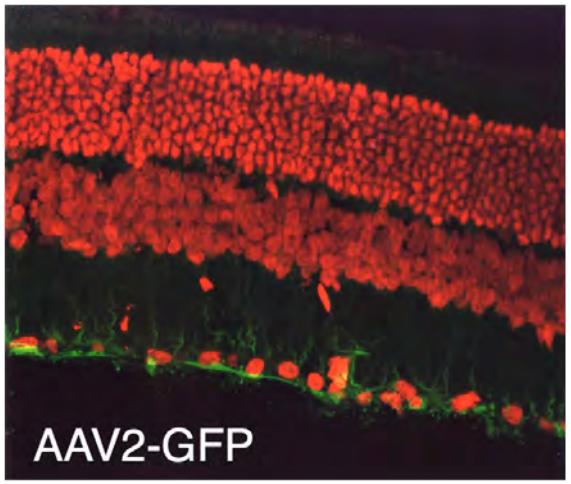
Heart



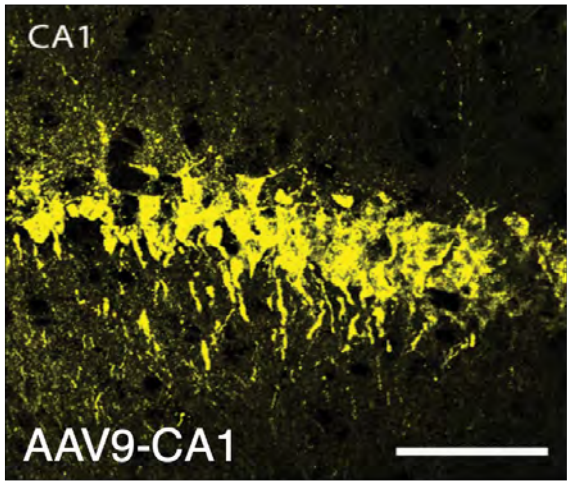
Cardiomyocytes



Brain

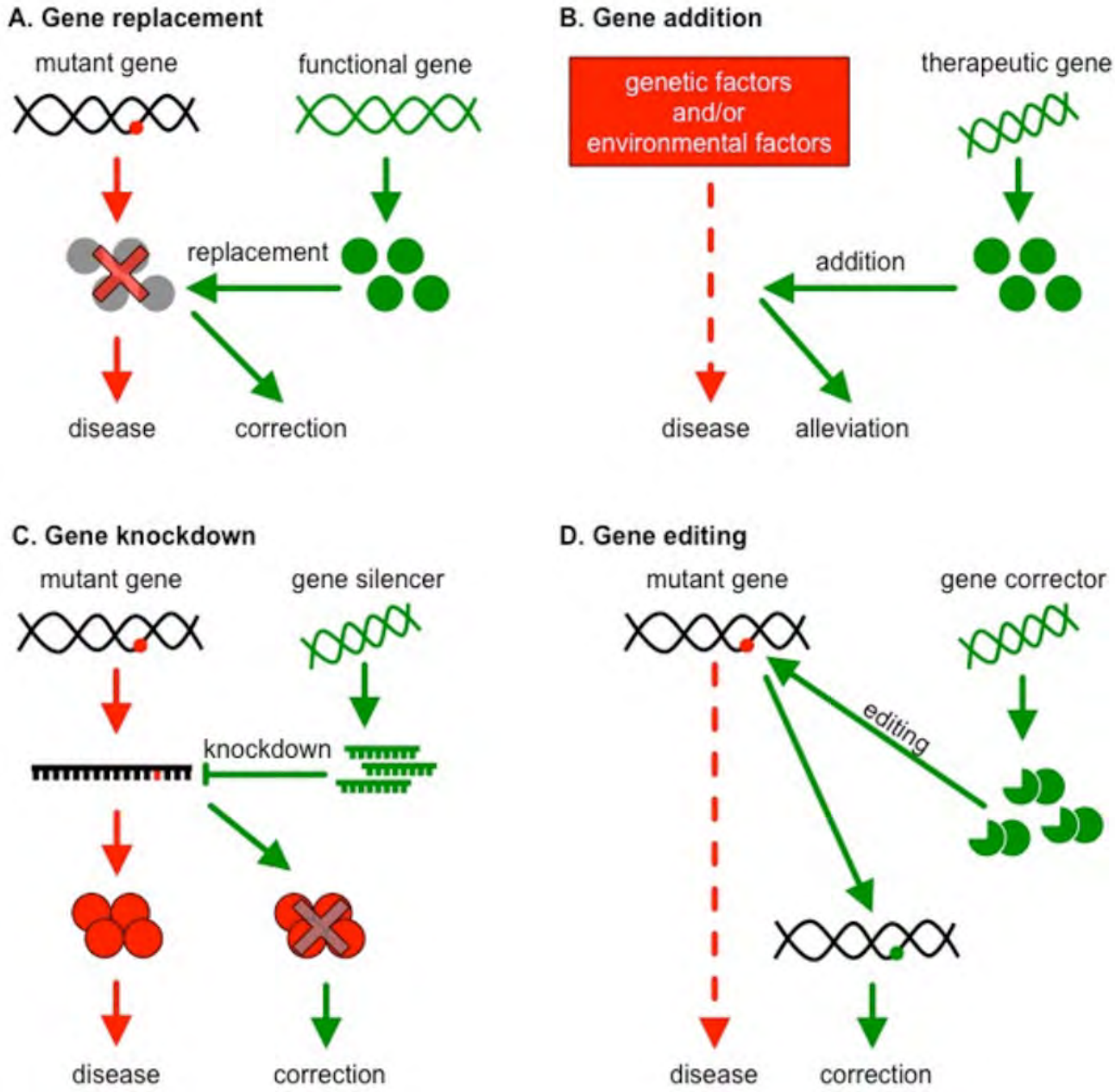


Retina

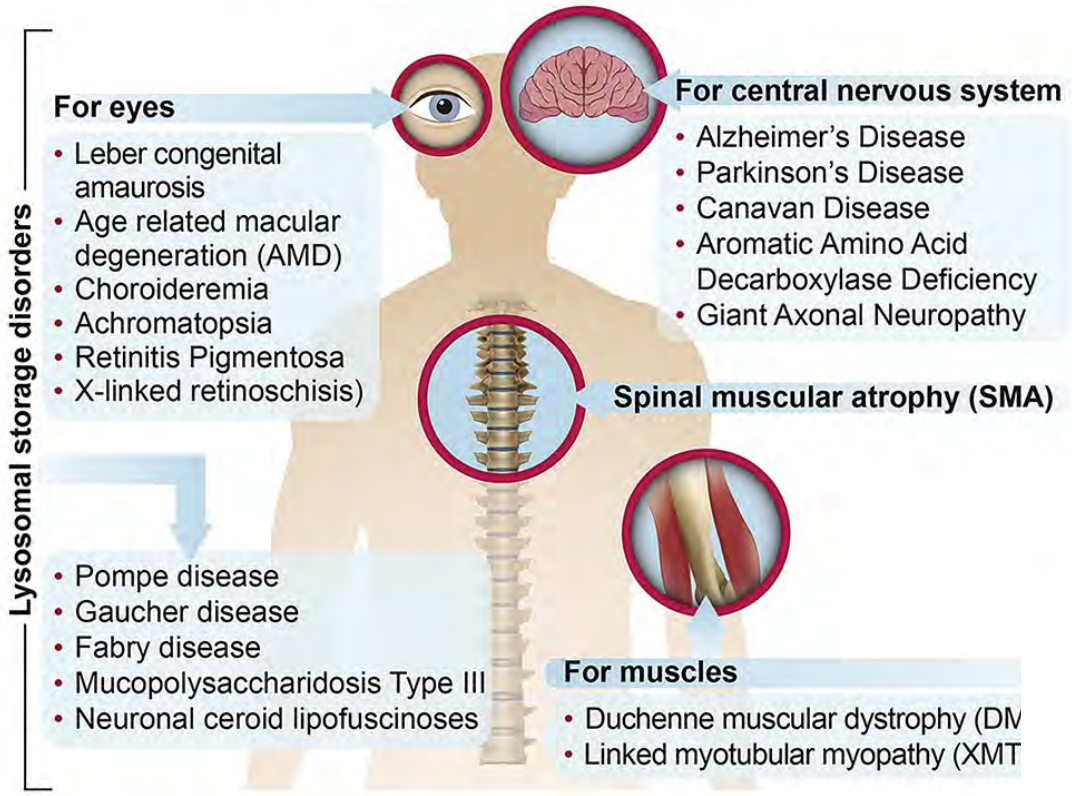


Neurons

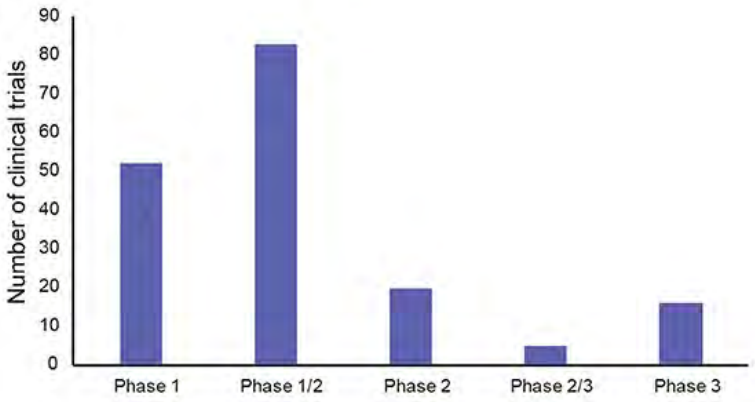
Four Gene Therapy Strategies



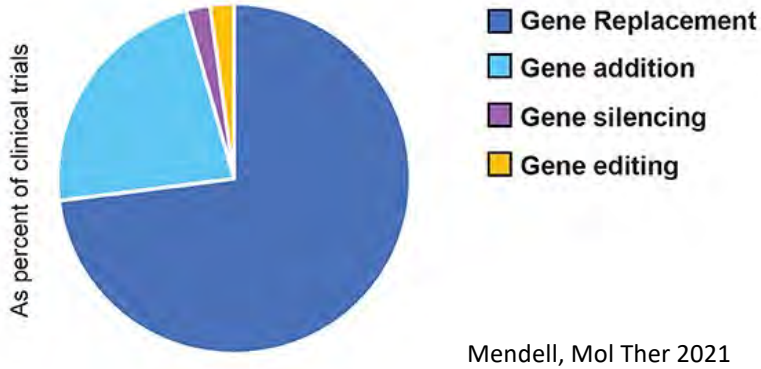
In vivo Gene Therapy with AAVs



Current status of the clinical phase



Gene Therapy Approaches



Mendell, Mol Ther 2021

Gene therapy AAV products that have obtained the approval by FDA in USA and by Ema in Europe

Name	Marketing Authorization Holder	Disease	Viral Vector
GLYBERA Alipogene tiparvovec	UniQure Netherlands	LPLD Lipoprotein lipase Deficiency	AAV1
LUXTURNA Voretigene neparvovec	Spark Eupharma	RPE65 mutation-associated blindness	AAV2
ZOLGESMA Onasemnogene abeparvovec	Novartis	SMA spinal muscular atrophy	AAV9
UPSTAZA Eladocagene exuparvovec	PTC Therapeutics International Limited	Severe deficiency of aromatic L-amino acid decarboxylase (AADC)	AAV2
HEMGENIX Etranacogene dezaparvovec	CSL Behring LLC.	Haemophilia B (congenital Factor IX deficiency).	AAV5
ROCTAVIAN Valoctocogene roxaparvovec	BioMarin International Limited.	Haemophilia A (congenital Factor VIII deficiency)	AAV5

Peculiarities of the eye as a target for gene therapy

The eye is a site of immune-priviledge

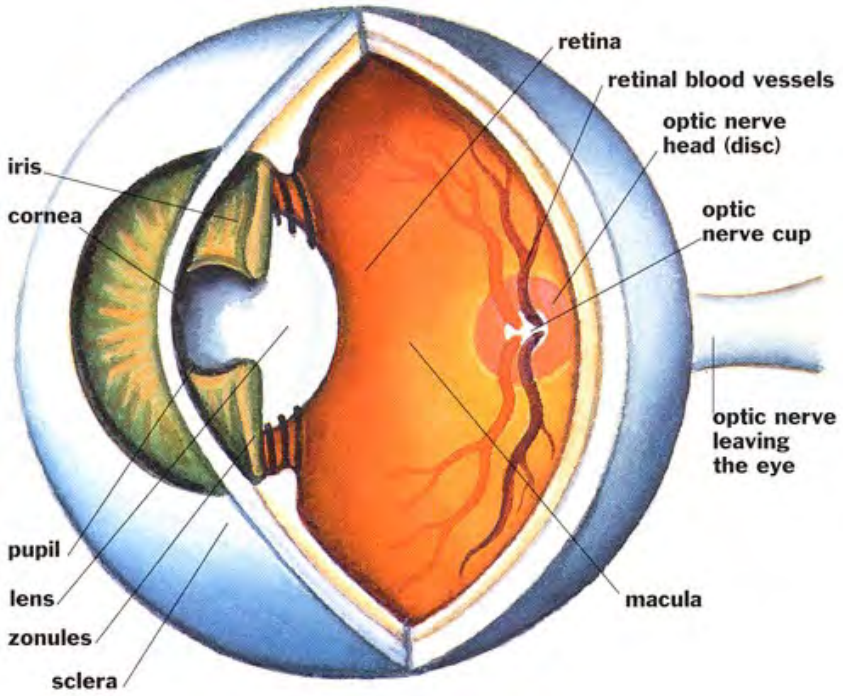
Most cells in the post-natal eye are terminally differentiated and prone to degenerative processes

Its compartmentalized anatomy (blood-retina barrier) enables local vector delivery in small volume with low likelihood of systemic dissemination

The eye is readily accessible for *in vivo* assessment by optical imaging and electrophysiological techniques

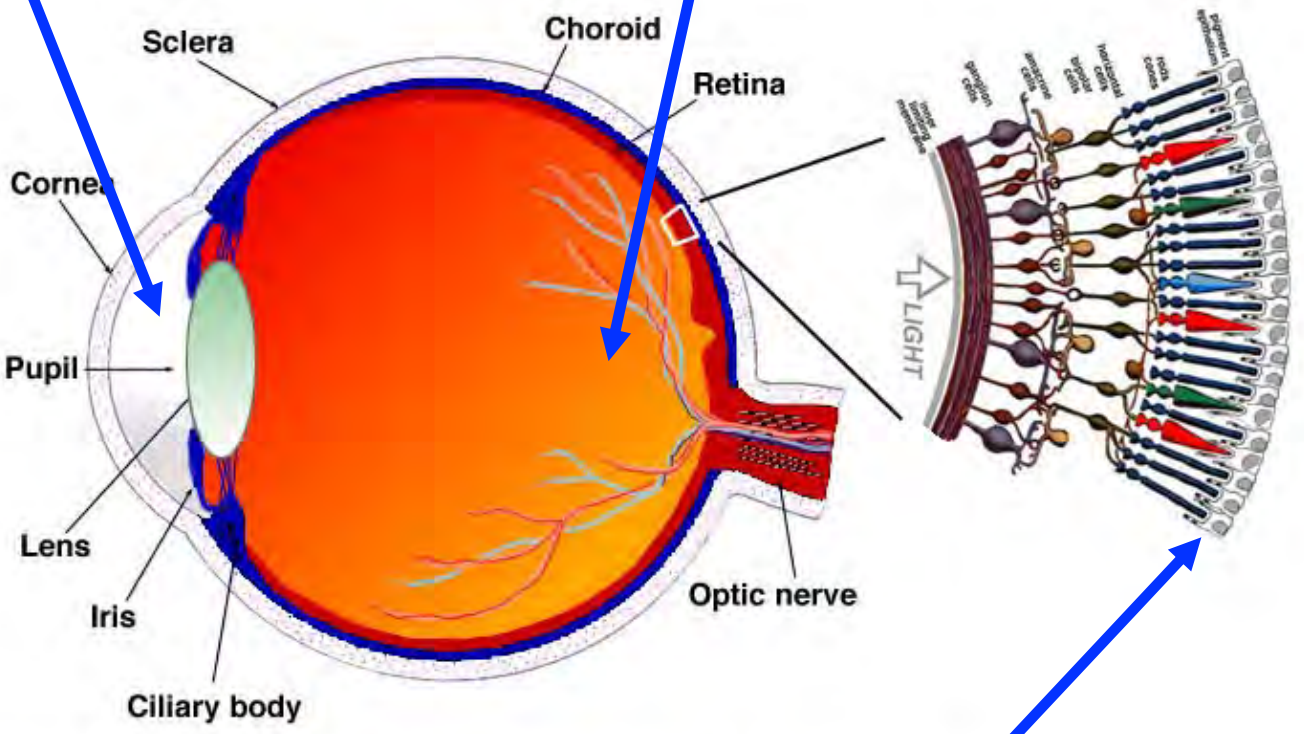
The results of the first clinical trials for ocular cancer and angiogenic disease have now been reported. One trial of gene replacement therapy for inherited retinal degeneration commenced recently and further such trials are expected to begin imminently

There are many animal models available



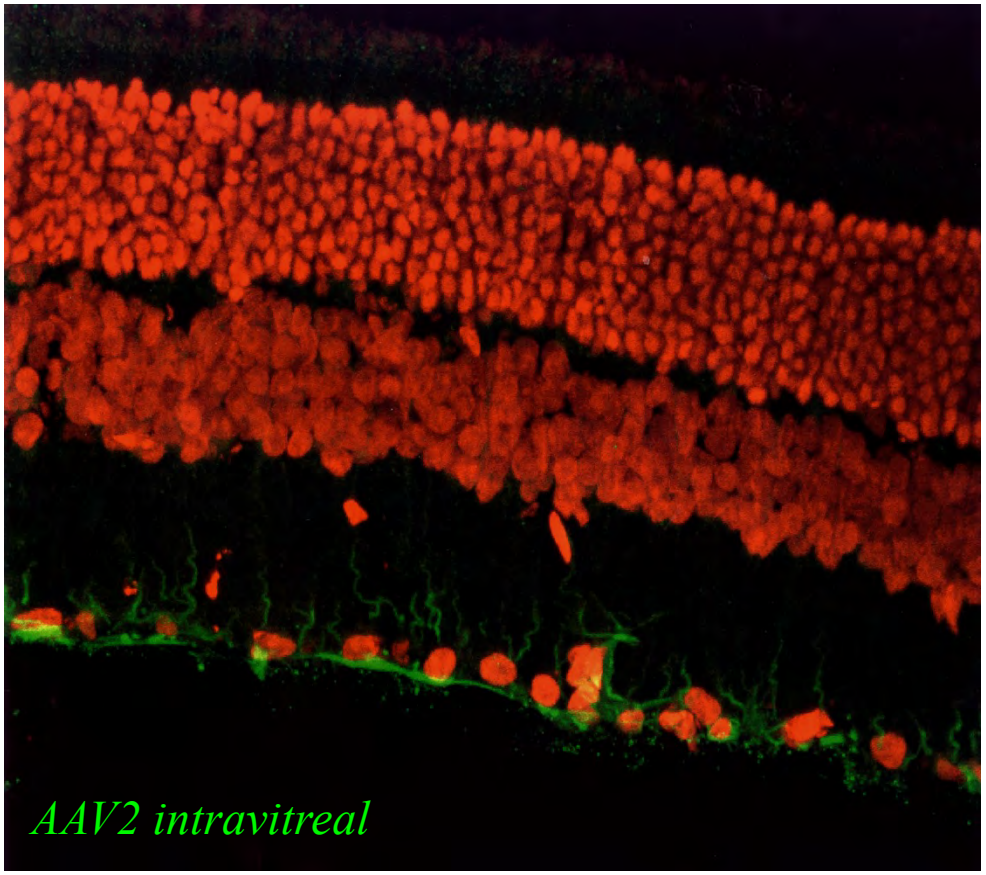
Into the anterior chamber - for corneal disease

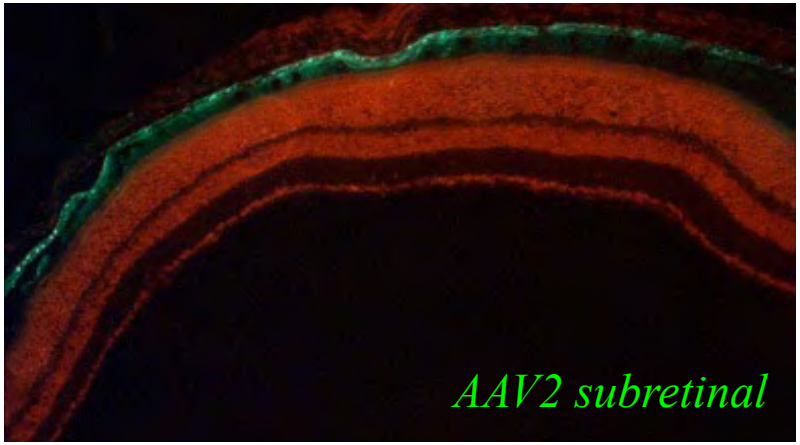
Intravitreal - anti-apoptotic or neurotrophic genes to protect RGC death in glaucoma

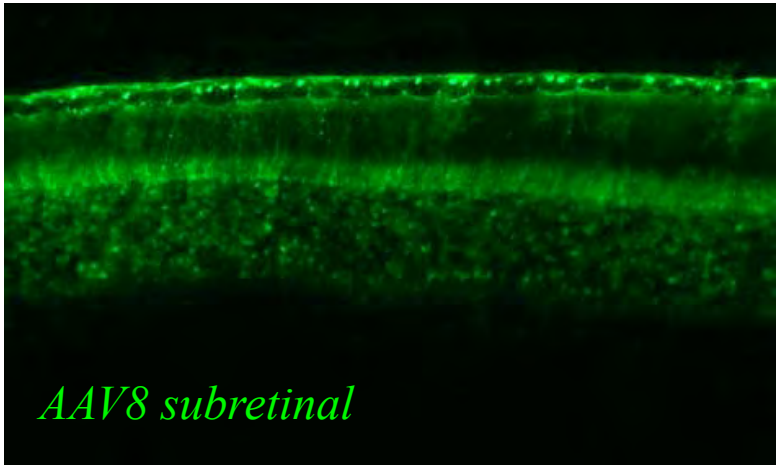


Sub-retinal (between photoreceptors and RPE) - for inherited retinal disorders, retinoblastoma and retinal neovascularization

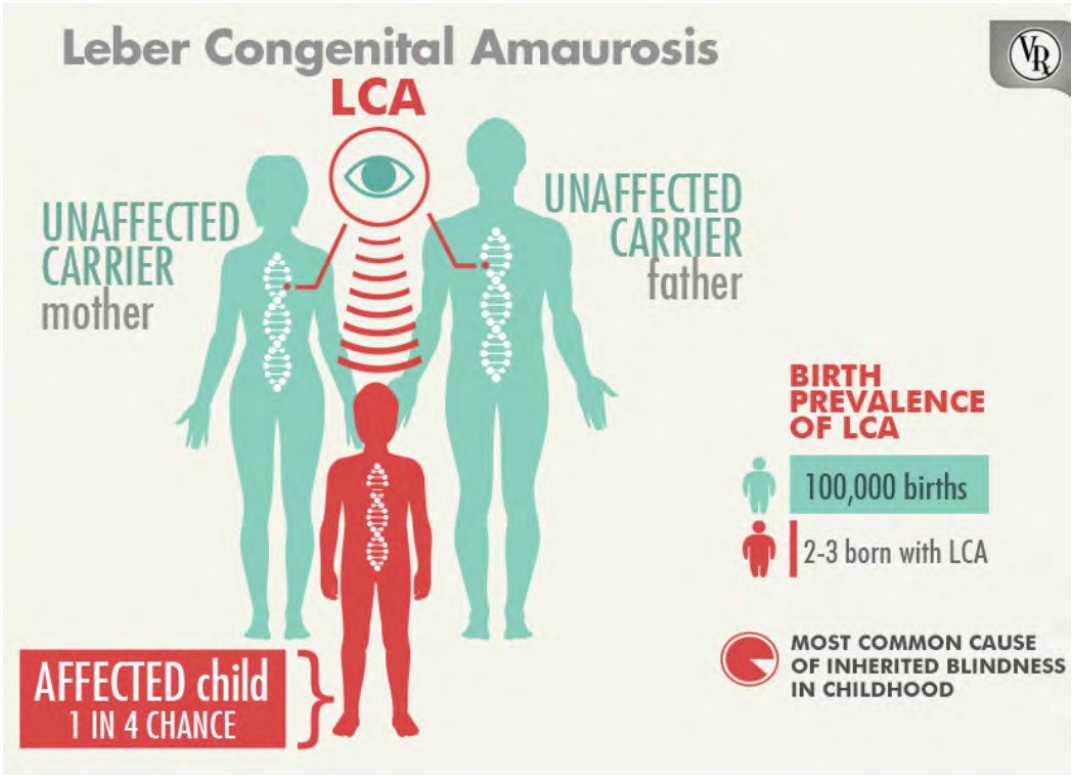
AAV is the only vector to efficiently transduce both RPE and photoreceptors

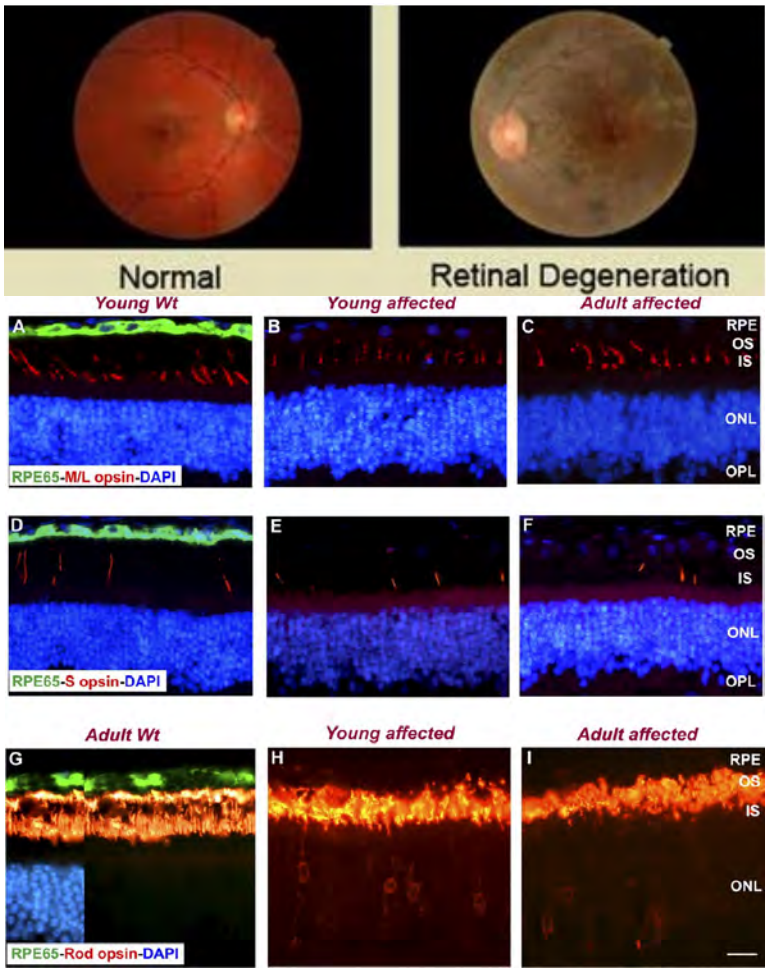






Leber Congenital Amaurosis

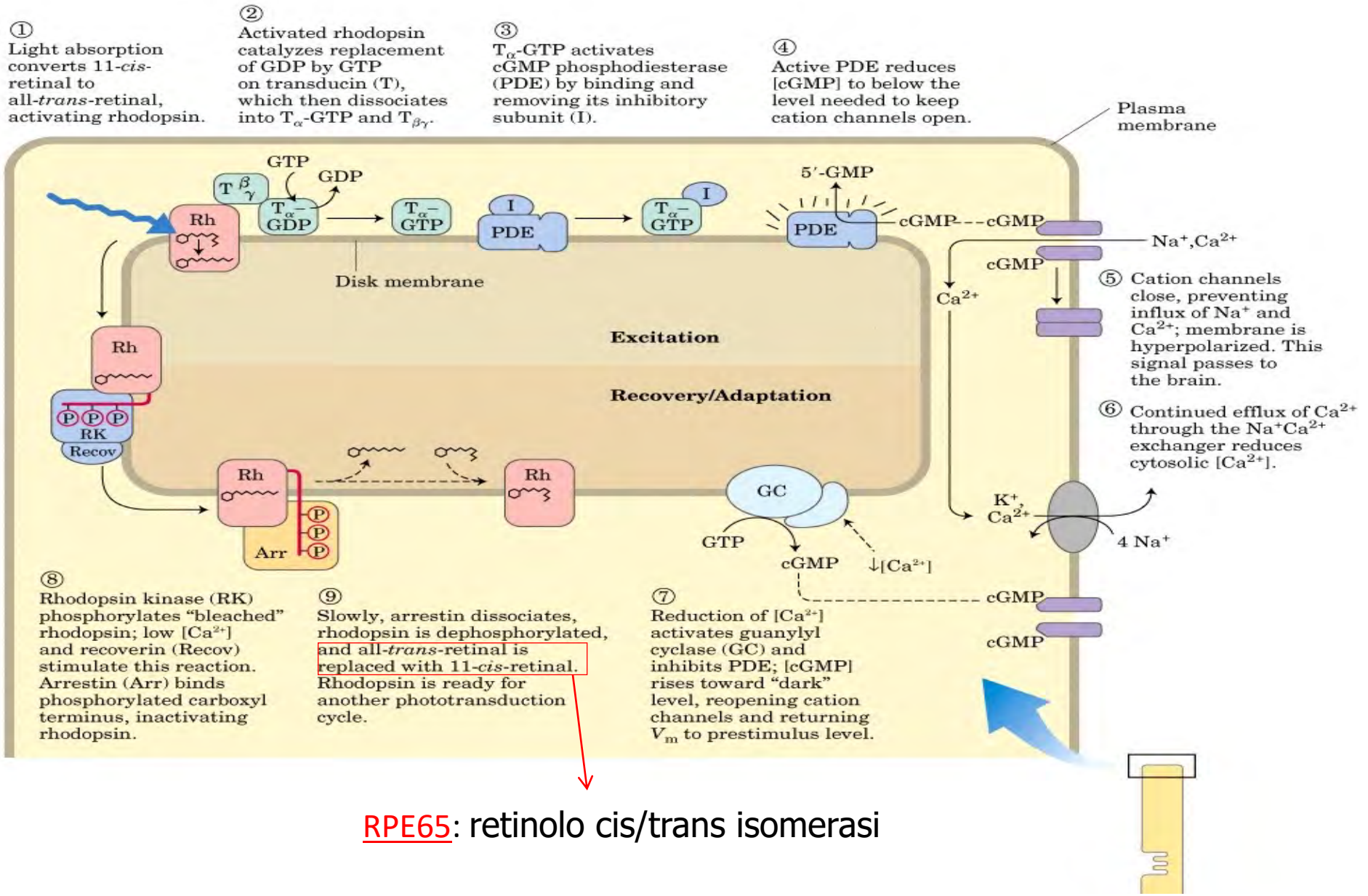




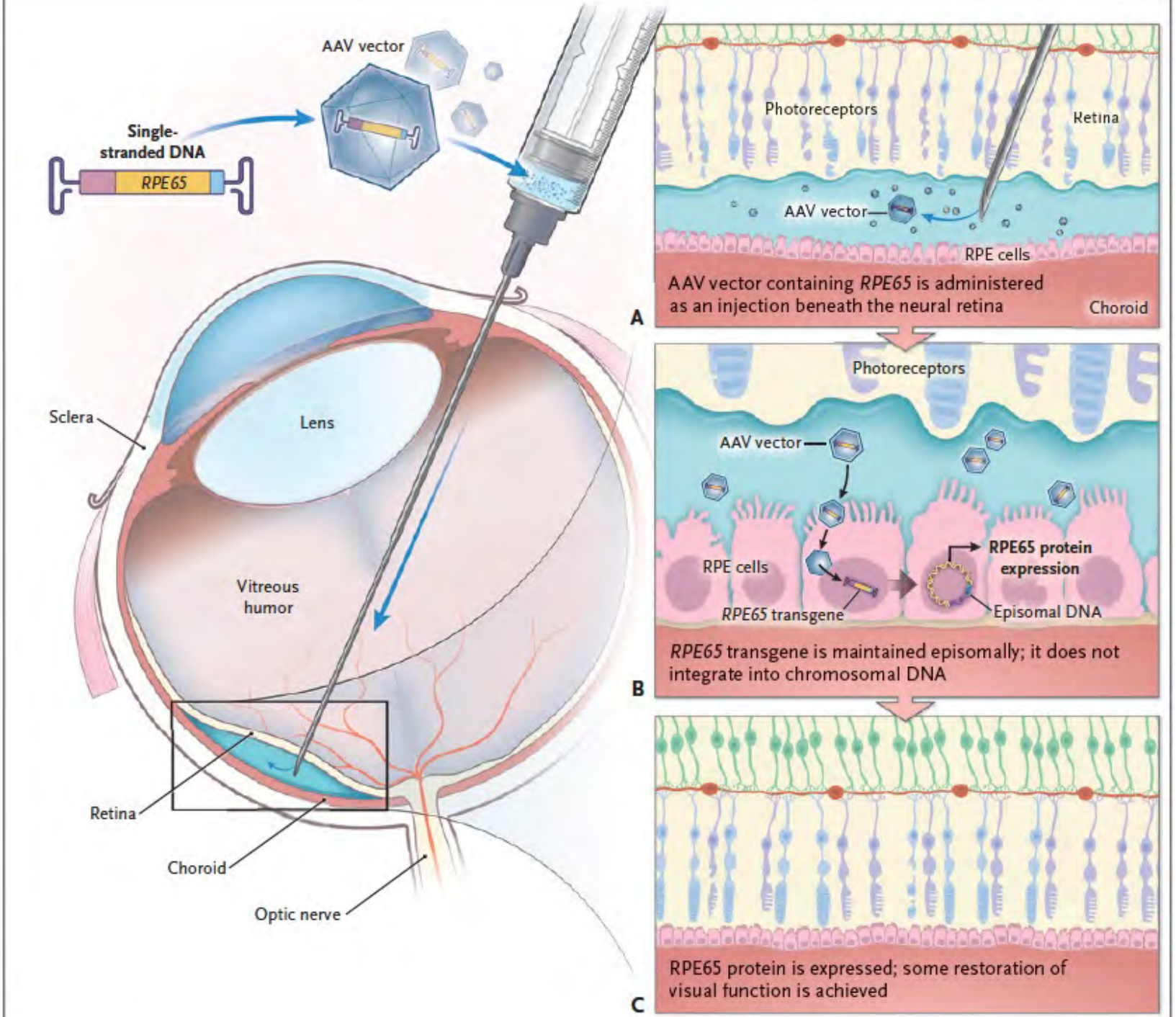
Vision loss in IR patients is due to the dysfunction/degeneration of retinal photoreceptor cells (PRs; rods and cones) and/or the retinal pigment epithelium (RPE)

Mutations in more than a dozen genes can cause LCA and *RPE65*-LCA is thought to represent about 6% of all LCA cases

Ciclo della fototrasduzione



RPE65: retinolo cis/trans isomerasi


AAV vector
Single-stranded DNA
RPE65
Sclera
Lens
Vitreous humor
Retina
Choroid
Optic nerve
Photoreceptors
Retina
AAV vector
RPE cells
AAV vector containing RPE65 is administered as an injection beneath the neural retina
Choroid
A
Photoreceptors
AAV vector
RPE cells
RPE65 protein expression
RPE65 transgene
Episomal DNA
RPE65 transgene is maintained episomally; it does not integrate into chromosomal DNA
B
RPE65 protein is expressed; some restoration of visual function is achieved
C

First AAV-Delivered Gene Therapy for Inherited Disease Approved by FDA

Luxturna is a recombinant adeno-associated virus of serotype 2 (rAAV2) expressing hRPE65. It is indicated for the treatment of patients with biallelic RPE65 mutation-associated retinal dystrophy.

One caveat: the injections cost $850.000 USD for patient (both eyes), making it one of the most expensive treatments in the world

> *In 41 patients in the clinical program, a single dose of Luxturna restored functional vision in these patients—and in a way that they were now able to conduct activities of daily living independently. The latest data, presented at the American Academy of Ophthalmology, suggests that one dose at three years and counting is still showing a sustained effect.*

- 30 patients treated in four independent trials
- The procedure was safe
- All trials showed significant improvements of both retinal and visual function
- Significant mobility improvement in navigation tasks
- The functional gain has been durable for as long as 3 years

Target disease	Vector	Sponsor	Phase
Leber congenital amaurosis 2	AAV2-hRPE65v2	Spark Therapeutics	I/II (follow on)
	AAV5-OPTIRPE65	MeiraGTx UK II Ltd	I, II
	AAV2-hRPE65v2-301	Spark Therapeutics	III
	AAV2-hRPE65v2-101	Spark Therapeutics	I/II
	AAV5-OPTIRPE65	MeiraGTx UK II Ltd	I/II
	AAV2.hRPE65p.hRPE65	University College, London	I/II, completed
	AAV2-hRPE65	Applied Genetic Technologies Corp	I/II, completed
	AAV2-hRPE65	University of Pennsylvania	I
	AAV2-hRPE65	Hadassah Medical Organization	I
	AAV4-hRPE65	Nantes University Hospital	I/II, completed

ZOLGENSMA

CORRIERE DELLA SERA / CRONACHE

LA VICENDA

Sofia, la bambina di Napoli curata con il farmaco più costoso al mondo

Ha 6 mesi e una malattia rara: per la terapia spesi 1,9 milioni. Il papà: «Per noi era un tunnel senza fine, finalmente ora possiamo sperare di vedere la luce»

di **Fulvio Bufi**

FARMACIA E BUSINESS

Novartis, il farmaco Zolgensma (atrofia spinale) diventa il più caro al mondo: costa 2,1 milioni di dollari

di **Redazione Economia** | 25 mag 2019

LOTTA ALL'EVASIONE

Lotteria degli scontrini, come funziona: dal codice per giocare alle estrazioni

LE AGEVOLAZIONI

Rottamazione, «saldo e stralcio»: nuove scadenze per 1,2 milioni di italiani

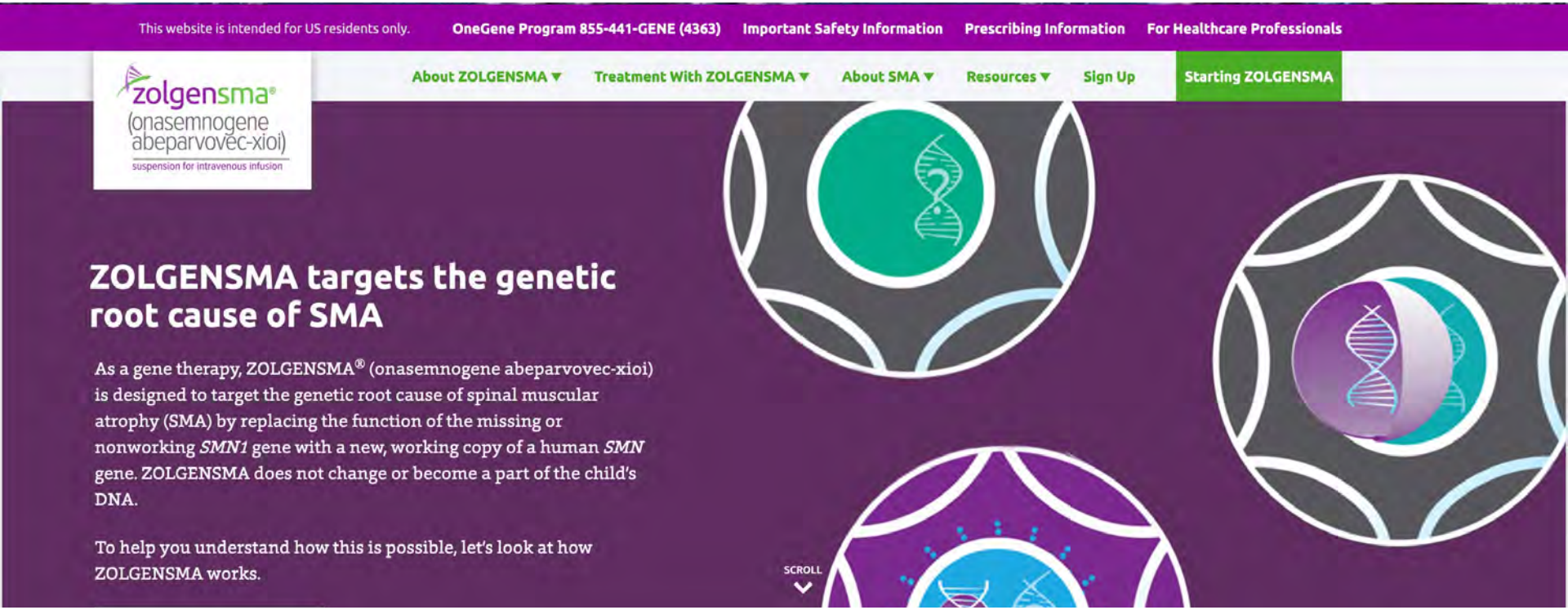
PAGAMENTI DIGITALI

Arriva il Cashback senza «Spid» e senza «App Io». Come fare per ottenerlo

L'INDAGINE

Smart working: così l'azienda può monitorare i dipendenti

7 dicembre 2020



What is ZOLGENSMA?

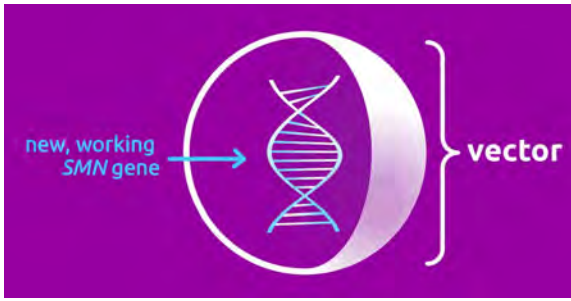
ZOLGENSMA is a prescription gene therapy used to treat children less than 2 years old with spinal muscular atrophy (SMA). ZOLGENSMA is given as a one-time infusion into a vein. ZOLGENSMA was not evaluated in patients with advanced SMA.

A targeted approach

ZOLGENSMA targets the genetic root cause of SMA by replacing the function of the missing or nonworking gene, called the *SMN1* gene. This gene is critical to making SMN protein.

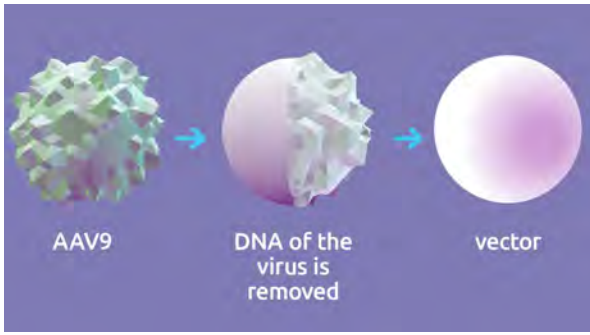
The importance of SMN protein

SMN protein is essential to motor neuron cell survival. These cells control muscle function. Without SMN protein, motor neuron cells die, causing muscles to become so weak that breathing, eating, and moving become difficult, and the condition is likely to become life threatening in its most severe forms.



The role of the vector

ZOLGENSMA is made up of a new, working copy of a human *SMN* gene that is placed inside a vector. A vector's job is to deliver the new, working *SMN* gene to the motor neuron cells in the body.



Delivery of the *SMN* gene

The vector that delivers the *SMN* gene is made from a virus called adeno-associated virus 9, or AAV9. This type of virus does not make people sick. To make the vector, the DNA of the virus is removed so that the new *SMN* gene can be put inside. Vectors are used because they can travel throughout the body and deliver the new, working gene to the cells where it is needed.

Production of SMN protein

When the new, working gene reaches its destination, it is ready to tell the motor neuron cells to start making SMN protein. This happens throughout the body, with many vectors delivering a new, working copy of the *SMN* gene to motor neuron cells. The new gene does not become part of the child's DNA.

Motor neuron cells maintained

With the motor neuron cells now able to make sufficient SMN protein, motor neuron cells that have not died may survive, function, and be maintained.

2022, 7 ottobre: Atrofia muscolare spinale di tipo 1: prima terapia genica in FVG effettuata dell'equipe servizio Malattie rare del Burlo

Il bimbo trattato è arrivato da noi a quattro mesi di vita con malattia già in stadio avanzato e grave compromissione di deglutizione e respiro. Dopo il trattamento, il decorso della malattia è stato bloccato e il bimbo anche se avrà bisogno di una carrozzina elettronica e dovrà essere sottoposto a ventilazione notturna, non è più in pericolo di vita.

Come spiegano i membri dell'equipe del servizio malattie rare del Burlo, la terapia genica in questione è relativamente semplice, poiché si tratta dell'infusione per via endovenosa di un vettore virale adeno-associato (denominato AAV9) che trasporta il gene mancante SMN1 nel sistema nervoso centrale.

Si tratta, inoltre, di una terapia innovativa e ancora altamente costosa (oltre un milione di euro).

ZOLGENSMA FOR SPINAL MUSCULAR ATROPHY

SMA is most often **LETHAL** in childhood

90% rate of death or permanent ventilation at two years of age in children with type 1 SMA

ALL of the children were **ALIVE** at two years in the pivotal trial of Zolgensma

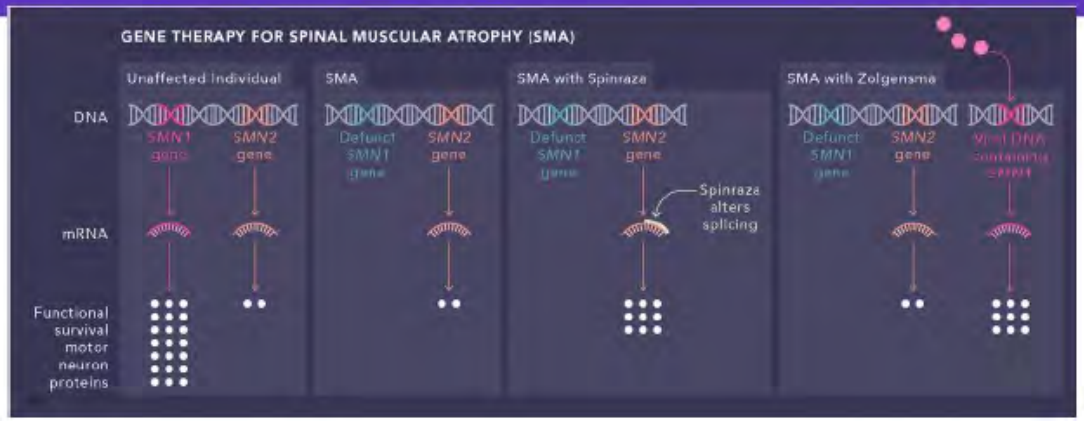
Price: $425,000 annually for five years = $2.125 million

Spinraza: The first ever drug for SMA, but dosed every four months intrathecally

Risdiplam & reldesemtiv: two potential competitors currently in phase II/III trials

Zolgensma: the second SMA market entrant, but the first ever SMA gene therapy. Only requires a single intravenous dose

Cortellis



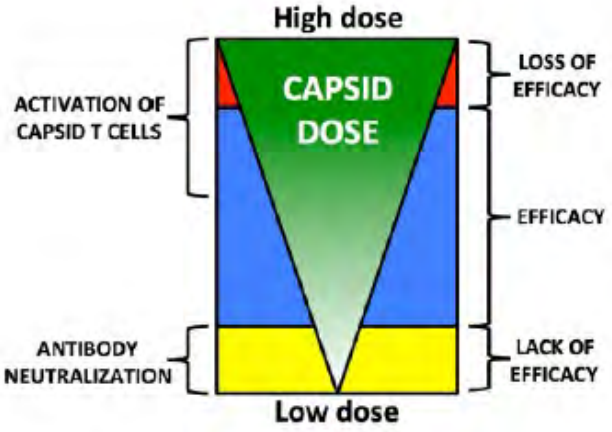
Long-Term Safety and Efficacy of Factor IX Gene Therapy in Hemophilia B

Amit C. Nathwani, M.B., Ch.B., Ph.D., Ulreke M. Reiss, M.D., Edward G.D. Tuddenham, M.B., B.S., Cecilia Rosales, Ph.D., Pratima Chowdary, M.B., B.S., Jenny McIntosh, Ph.D., Marco Della Peruta, Ph.D., Elsa Lheriteau, Ph.D., Nishal Patel, F.R.C.P., F.R.C.Path., Deepak Raj, M.B., B.S., Ph.D., Anne Riddell, B.Sc., Jun Pie, B.S.N., Savita Rangarajan, M.B., B.S., David Bevan, M.B., B.S., Michael Recht, M.D., Yu-Min Shen, M.D., Kathleen G. Halka, M.D., Etiena Basner-Tschakarjan, M.D. Ph.D., Federico Mingozzi, Ph.D., Katherine A. High, M.D., James Allay, Ph.D., Mark A. Kay, M.D., Catherine Y.C. Ng, M.S., Junfang Zhou, M.D., Maria Cancio, M.D., Christopher L. Morton, B.S., John T. Gray, Ph.D., Deokumar Srivastava, Ph.D., Arthur W. Nienhuis, M.D., and Andrew M. Davidoff, M.D.
N Engl J Med 2014; 371:1994-2004November 20, 2014DOI: 10.1056/NEJMoa1407309

Dose-dependent increase in circulating factor IX to a level that was 1 to 6% of the normal value over a median period of 3.2 years, with observation ongoing

Reduction of more than 90% in both bleeding episodes and the use of prophylactic factor IX concentrate.

In 10 patients with severe hemophilia B, the infusion of a single dose of AAV8 vector resulted in long-term therapeutic factor IX expression associated with clinical improvement. With a follow-up period of up to 3 years, no late toxic effects from the therapy were reported.



It will cost **US$3.5 million** for each dose, making it the most costly medication in the world. At first look, the price appears exorbitant, but a new examination of the drug's cost-effectiveness reveals that the life-span cost of continuous infusion of FIX for each individual with mild to chronic hemophilia B is between US$21 and $23 million

AAV vectors as gene therapy tools – why the limited success?

IND application

FDA review

PRECLINICAL TRIALS

CLINICAL TRIALS (I, II, III, IV)

POST-MARKET SURVEILLANCE

***IN VITRO* STUDIES (Basic research)**

1. Target discovery
2. Target validation
3. Drug generation
4. Drug testing *in vitro*

***IN VIVO* STUDIES (Animal models)**

1. Dose range finding and delivery optimization
2. Proof of concept (safety and potency)
3. Toxicology

HUMAN TESTING

1. Healthy volunteers (safety, pharmacokinetic tolerability)
2. Patient population (safety and efficacy)

PHARMACOVIGILANCE

1. FDA approval
2. Drug registration
3. Long-term safety and efficacy

6 – 7 years

0.5 – 2 years (FDA)

Luxterna (2017)

Zolgensma (2019)

SUCCESSFUL DRUGS


Il doping dei geni cambierà la natura dello sport
Atleti geneticamente modificati
Quando il metano dominava il clima
Codice Voynich: truffa o mistero?
Rane, parassiti e nuove malattie
Numero 432 - agosto 2004 - € 3,90

WORLD ANTI-DOPING AGENCY

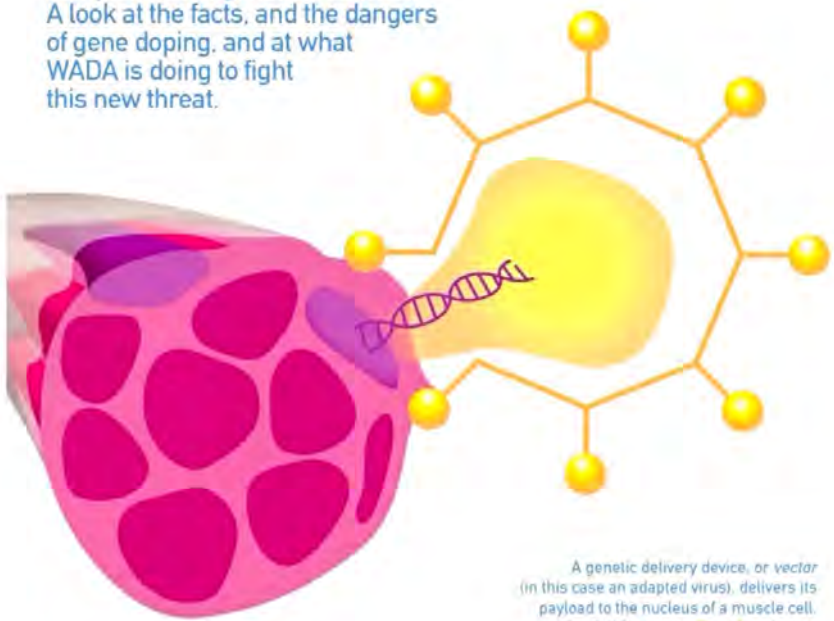
play true

ISSUE 1 - 2005

AN OFFICIAL PUBLICATION OF THE WORLD ANTI-DOPING AGENCY

Gene Doping

Science and sport converge once again as medical research charts the complexities of genetic treatment. A look at the facts, and the dangers of gene doping, and at what WADA is doing to fight this new threat.



A genetic delivery device, or *vector* (in this case an adapted virus), delivers its payload to the nucleus of a muscle cell. See full feature on Page 2 and more detailed explanation on Page 5.

Editorial: Richard W. Pound

01 Taking the Lead

Gene therapy represents an exciting and promising step forward in medical research, but its use to enhance athletic ability is as wrong as any type of traditional doping.

As the Olympic Games in Athens wrapped up last summer, I was frequently asked one question by journalists who were already thinking ahead to the Beijing Games: Could there be genetic doping by 2008?

The idea that genetically-altered athletes could be competing at the Olympics in Beijing is disturbing but not out of the realm of possibility. WADA has, for some time, considered genetic doping to be a looming threat. We have taken the lead in focusing the attention of the scientific and sport communities on the challenge this new method presents for enhancing performance. As you will read in the following pages, our scientists do not believe that genetic doping is a reality – yet. But that could very well change in the next few years and we have to be ready with new weapons in our arsenal to deal directly with this new threat.

In 2002, WADA brought together, for the first time, leaders in sport and in science to discuss the issue of gene doping at the Banbury Conference in New York. The conference was exactly what we needed to place gene doping on the map for those who could do something to prevent it before it ever gets off the ground. Those involved in sport learned just how far science has come in the field of gene therapy. Cures for many devastating diseases now sit tantalizingly within our reach. The scientists learned just how far some athletes will go to be the best. They heard directly from some of their colleagues who had already had calls from coaches and trainers, asking how gene therapy could be applied to their athletes solely to enhance performance.

It was an eye-opening event for all of us and led to the inclusion of gene doping as a prohibited method on the 2003 Prohibited List of Substances and Methods. Since that time, we have done quite a bit to get ahead of the cheaters in this field. We have partnered up with some of the best scientists in the world to fund research projects into how gene doping can ultimately be detected. We have created a gene doping panel, whose members will continually advise us on cutting edge technology in gene doping detection. And we have undertaken education efforts to let athletes and their entourages know that gene doping is still an imperfect science and quite dangerous. In addition to being as morally wrong as traditional doping, gene doping can present a significant risk to health.

Last winter, I spoke to the annual meeting of the American Association for the Advancement of Science on this topic. One point I stressed to them is the need for governments and regulatory bodies to create a framework for regulating the application of gene transfer technology. Some of that is already in place. In the U.S., for example, there are stringent rules regarding oversight of gene transfer studies. But there is still much that needs to be done in this area. Some disreputable labs would be willing to replicate the technology for performance enhancement – for the right price. As dangerous and wrong as traditional doping is, it is hard to conceive what the consequences could be of altering a person's genetic makeup just to make them better in sports. This is a slippery slope we do not ever want to go down.

We should all keep in mind one important point: gene therapy is an incredible step forward in the field of medicine and a testament to human ingenuity and ability. The fact that science now allows us to tinker with our genetic codes in order to be healthier human beings is a remarkable achievement. While we have a long way to go, I am confident science will get us to the point where gene transfer technology can be applied safely and effectively. But to misuse this advancement to create super athletes is not acceptable. WADA will fight gene doping as vigorously as it has traditional doping. Competitions should still be won through hard work, training, and dedication. ■

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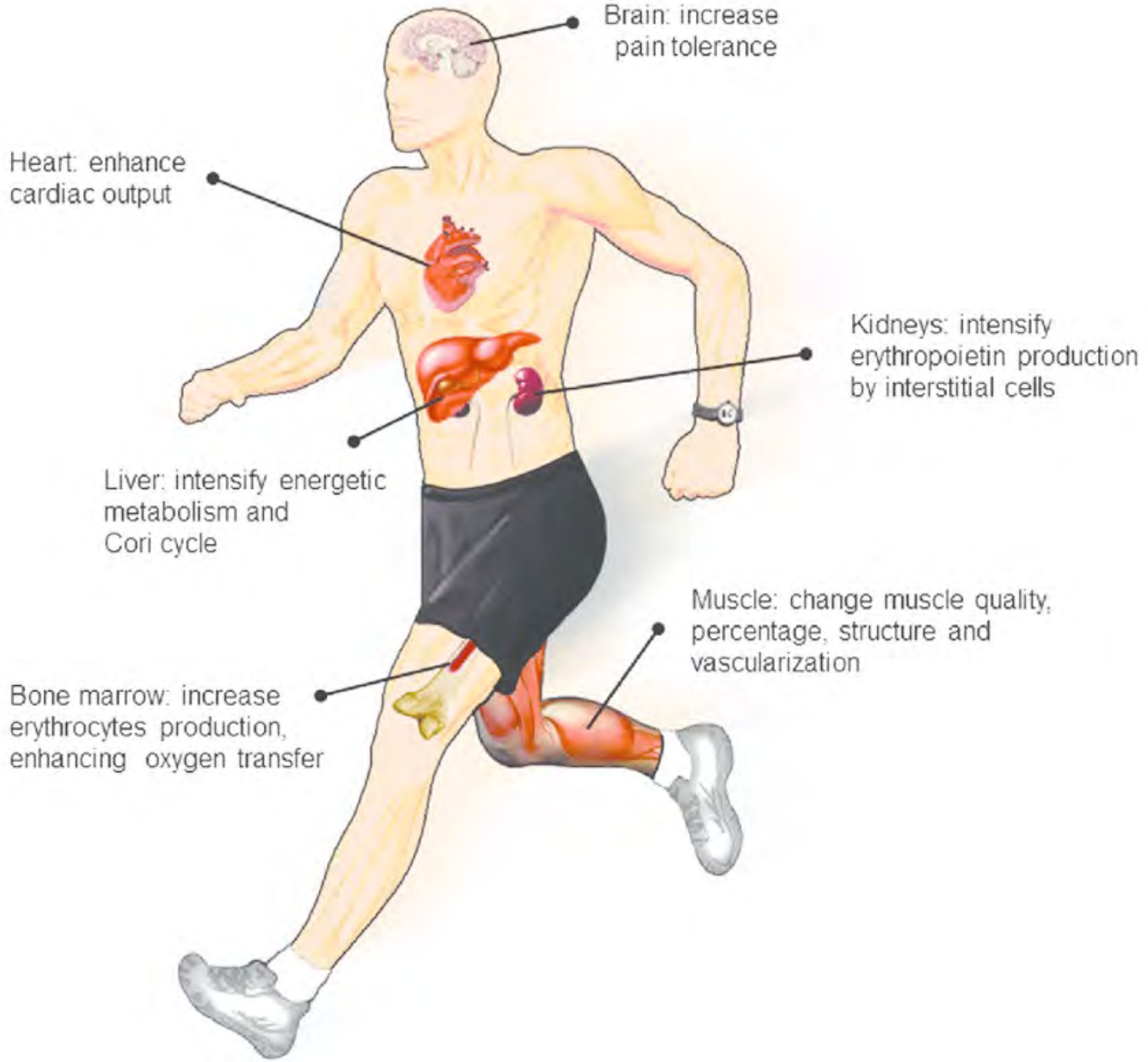
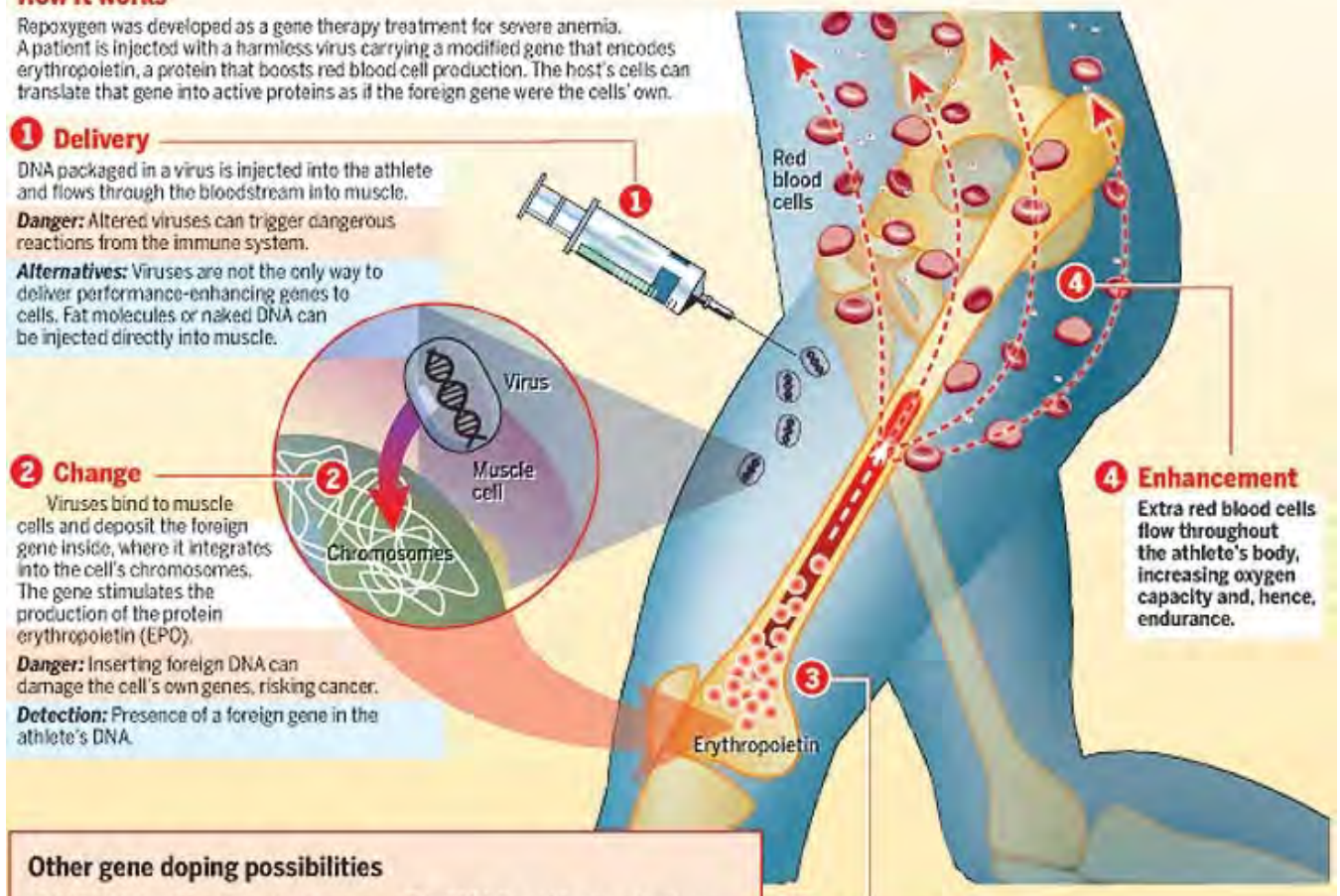

Brain: increase pain tolerance
Heart: enhance cardiac output
Kidneys: intensify erythropoietin production by interstitial cells
Liver: intensify energetic metabolism and Cori cycle
Muscle: change muscle quality, percentage, structure and vascularization
Bone marrow: increase erythrocytes production, enhancing oxygen transfer

Gene doping

Repoxygen is a new way to artificially enhance an athlete's performance — one that is hard to detect and with potentially permanent effects

How it works

Repoxygen was developed as a gene therapy treatment for severe anemia. A patient is injected with a harmless virus carrying a modified gene that encodes erythropoietin, a protein that boosts red blood cell production. The host's cells can translate that gene into active proteins as if the foreign gene were the cells' own.



Other gene doping possibilities

■ In 1988, H. Lee Sweeney and colleagues at the University of Pennsylvania School of Medicine injected mice with a virus carrying a gene that boosted production of insulin-growth factor 1 (IGF-1). The injected mice had 15% more muscle mass than untreated mice.

■ In 2004, Ronald Evans and colleagues at California's Salk Institute for Biological Studies engineered mice to have extra copies of the gene encoding a protein called peroxisome proliferator-activated receptor delta (PPAR-delta). These mice could run twice as far as unaltered mice.

SOURCE: *Finding the Golden Genes*, Patrick Barry, www.sciencenews.org

SUSIE MAH/ SUN MEDIA

WORLD ANTI-DOPING AGENCY

Geni:

-EPO
-IGF1
-Inibitori della Miostatina

Terapia genica: limiti e prospettive

Limiti attuali		Prospettive future
bassa efficienza di rilascio genico	⇨	sviluppo di nuovi vettori
bassa specificità di bersaglio	⇨	sviluppo di strategie cellulo-specifiche
espressione transiente e non-fisiologica	⇨	approcci di gene-targeting
reazione immunitaria contro i vettori	⇨	sviluppo di vettori non-immunogenici