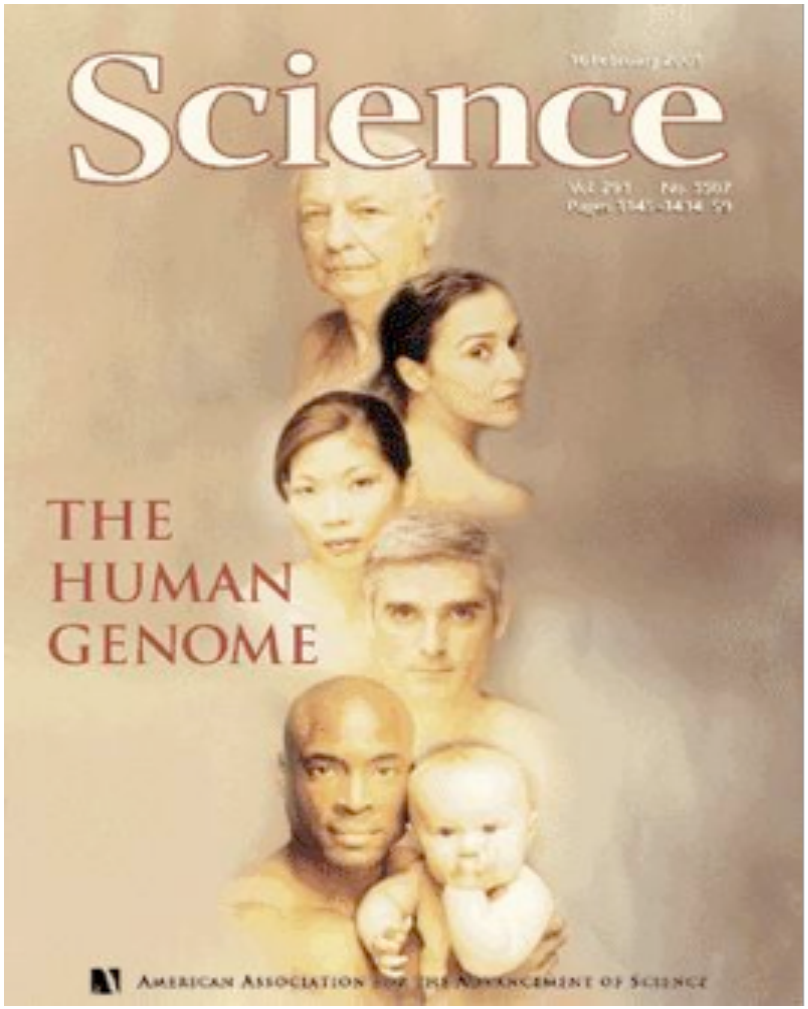
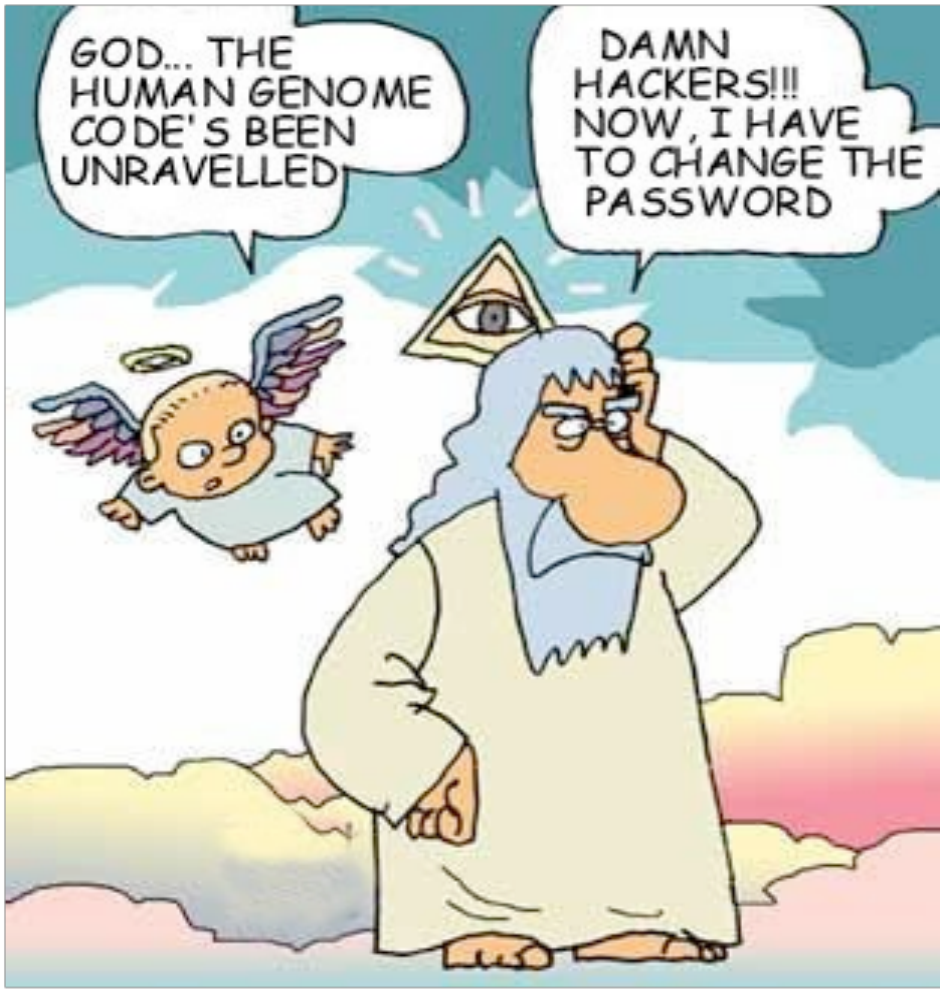
Ingegneria genetica e medicina: paure e perplessità





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?

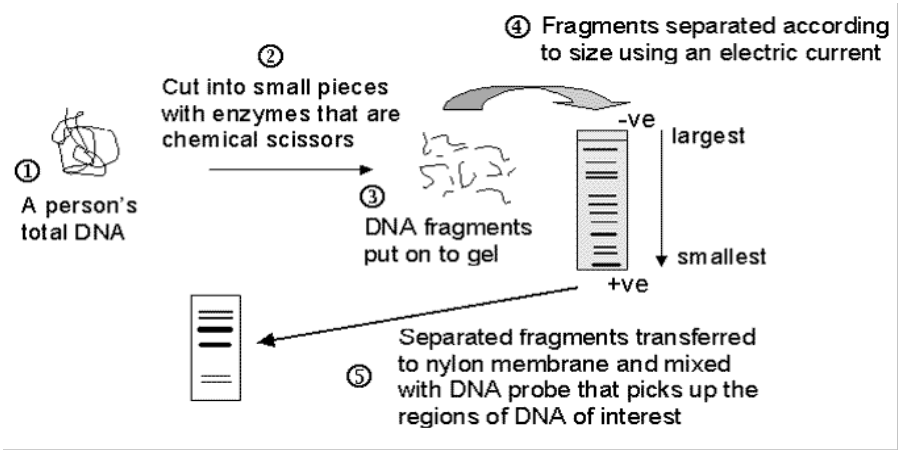
- Per la ricerca pura molto utili per comprendere a fondo la funzione di una determinata proteina
- Per la ricerca applicata, il fine ultimo è quello di conferire a determinati organismi caratteristiche importanti per svolgere determinati scopi.

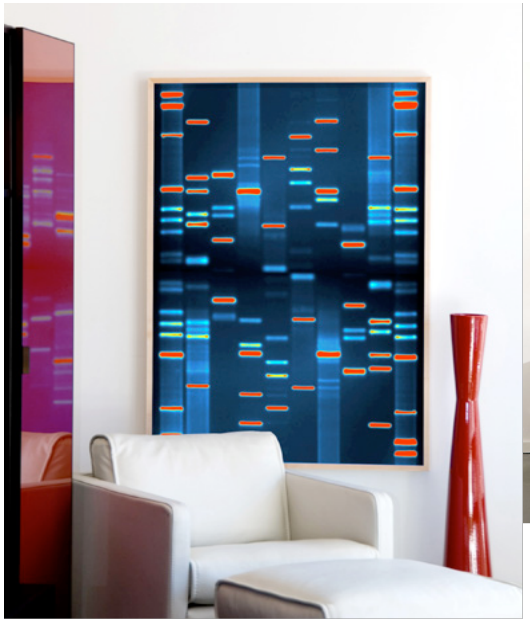
Agricolo: ad es. cereali resistenti agli erbicidi)

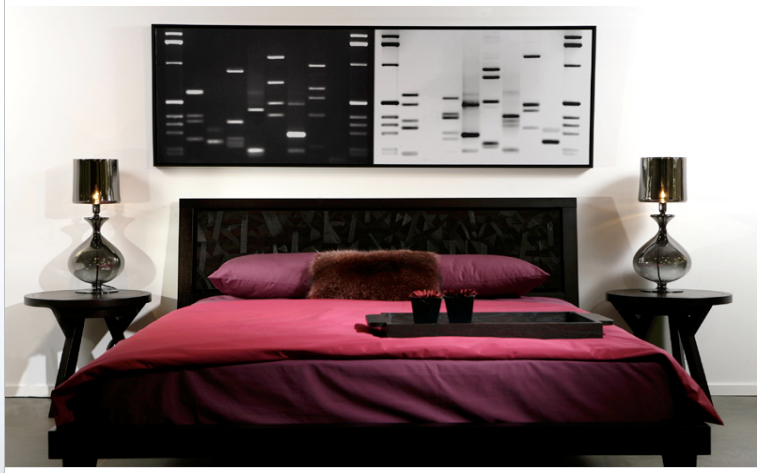
Biomedico: produzione di farmaci ricombinanti (ad es. la produzione di insulina attraverso batteri)

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





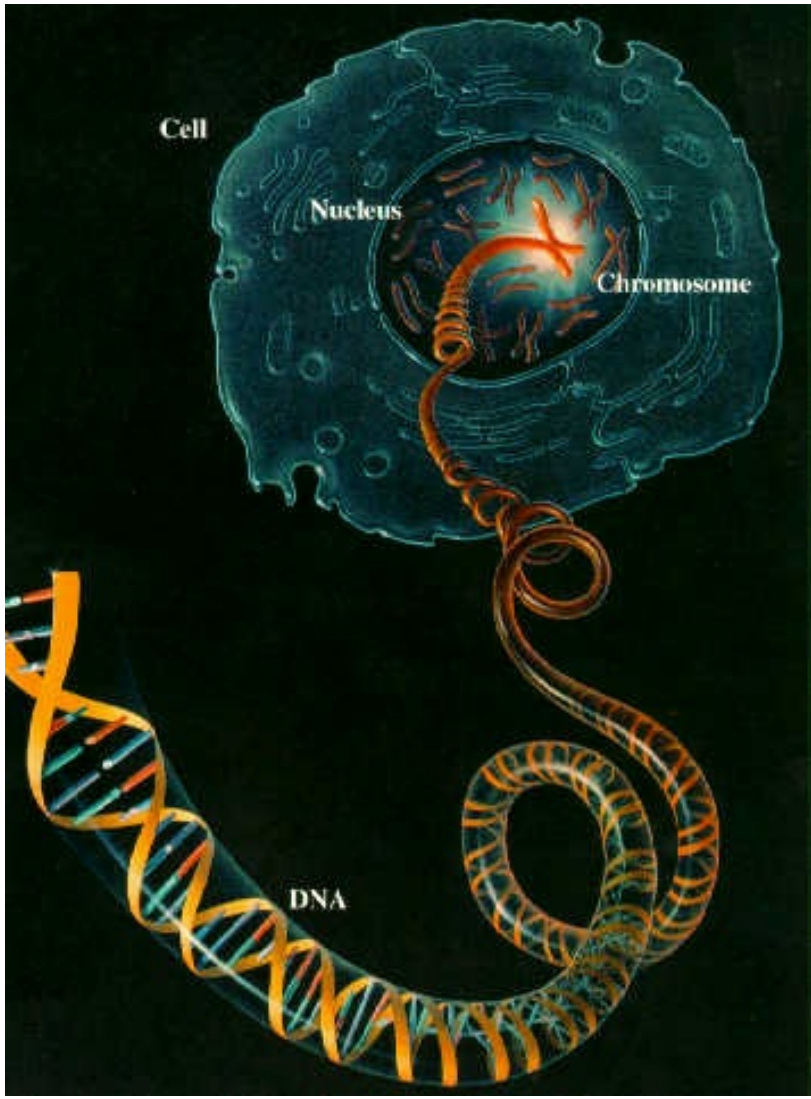


L’ingegneria genetica applicata all’uomo:

la terapia genica

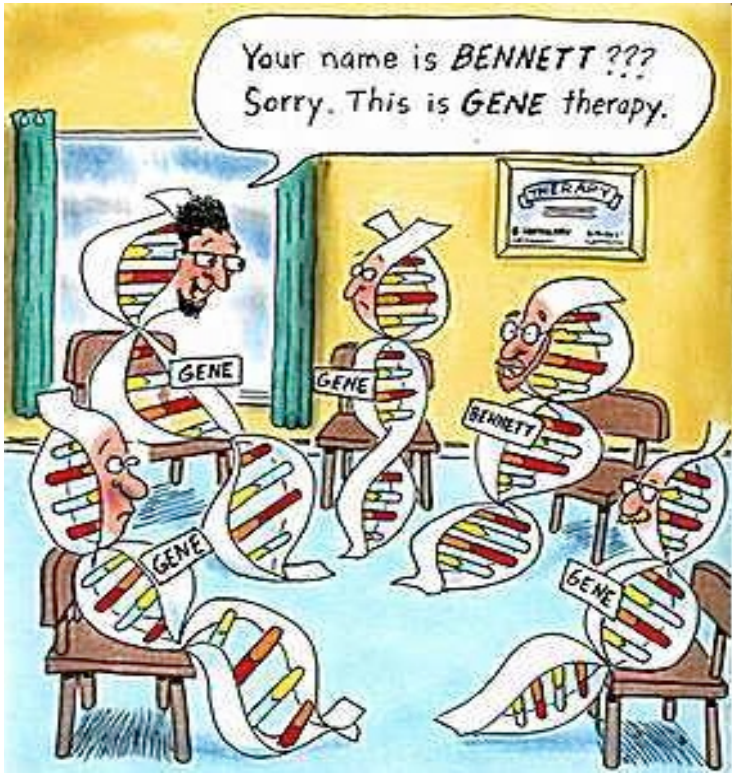
TERAPIA GENICA

- Introduzione di un transgene in una cellula allo scopo di correggere un errore innato del metabolismo 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 all GT

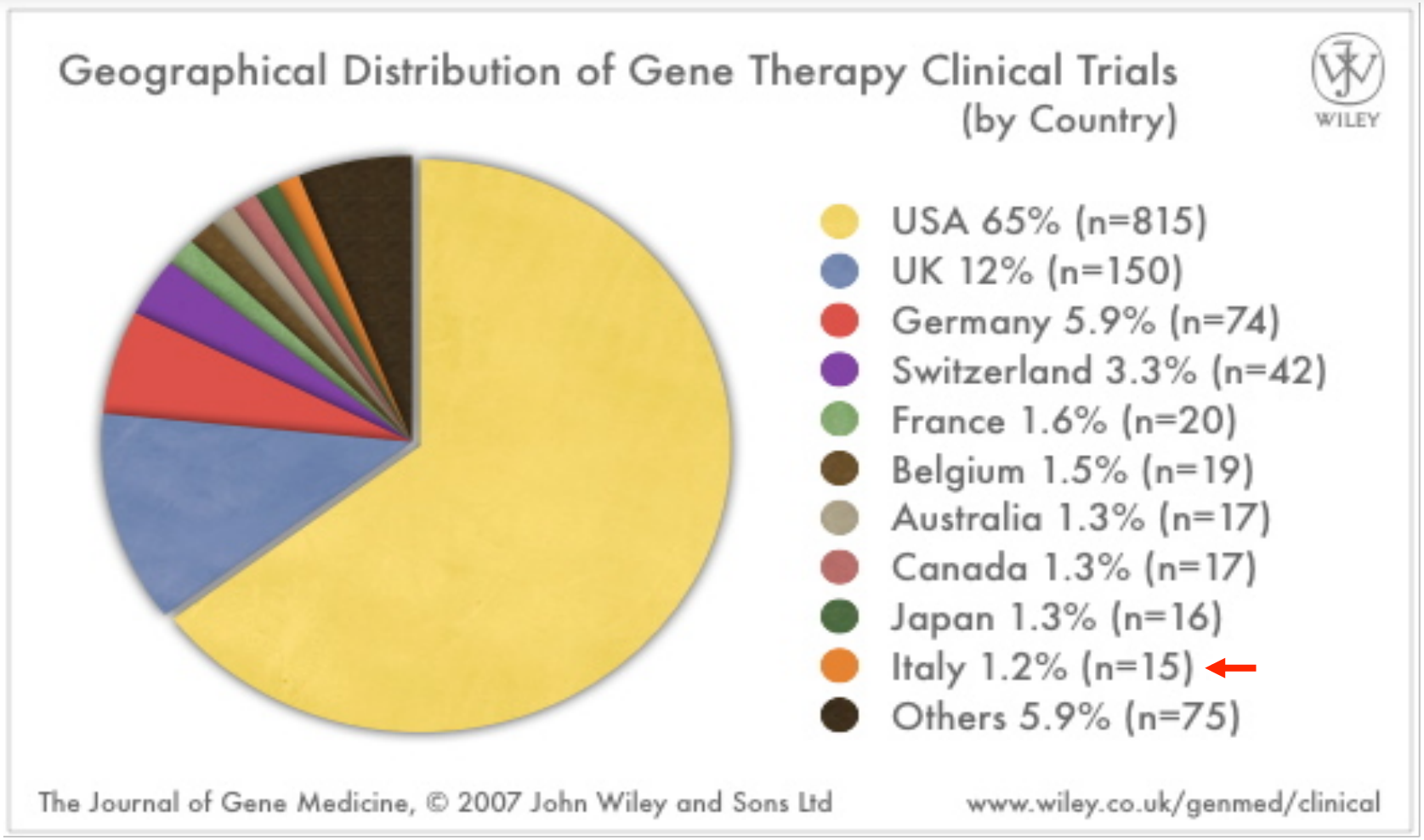
Disease	Defect	Incidence	Target Cells
Severe combined immunodeficiency (SCID)	Adenosine deaminase (ADA) in 25% of SCID patients	Rare	Bone-marrow cells or T lymphocytes
Hemophilia A	Factor VII deficiency	1:10,000 males	Liver, muscle, fibroblasts or bone marrow cells
Hemophilia B	Factor IX deficiency	1:30,000 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	

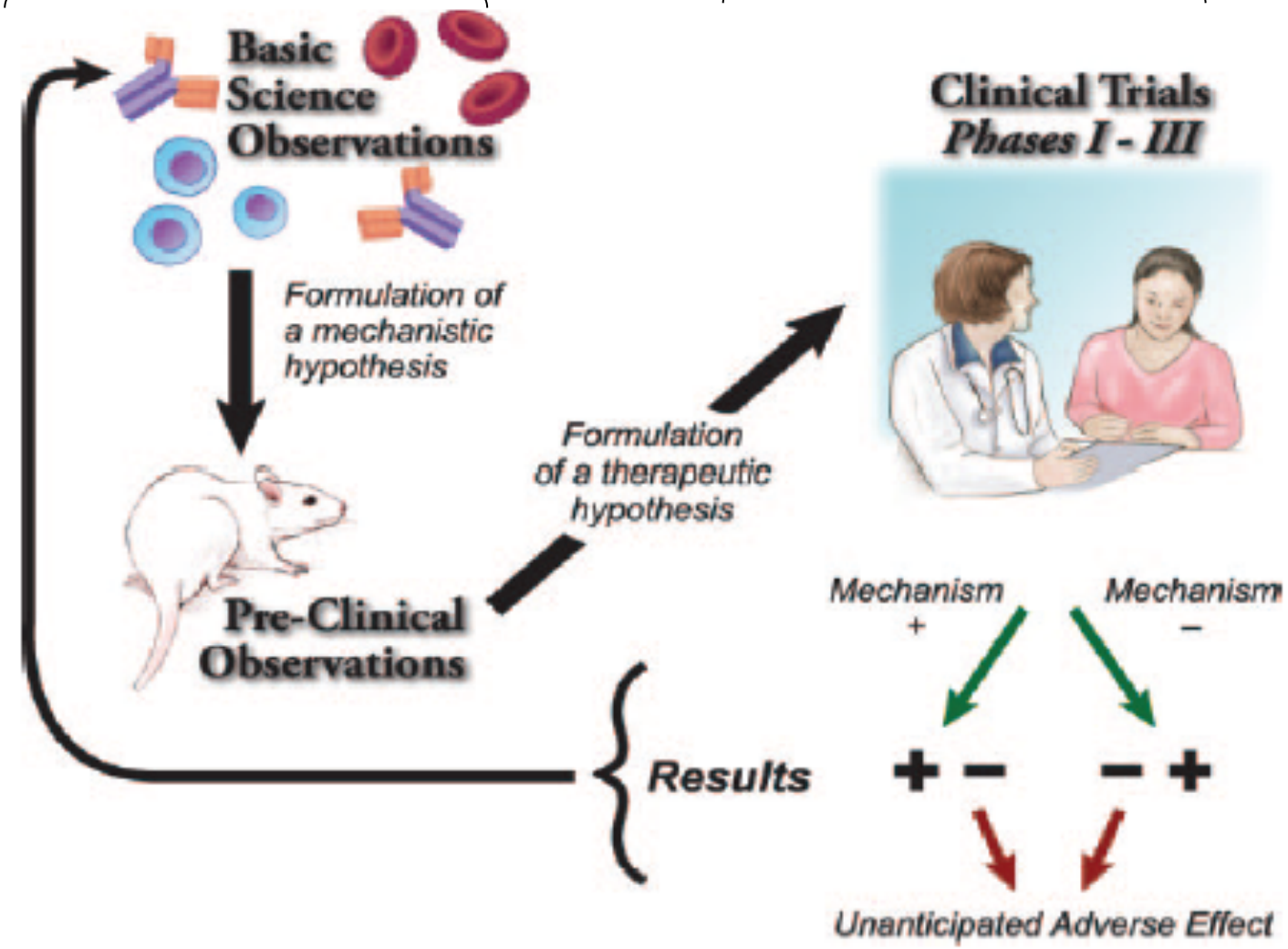
Chi investe in terapia genica?



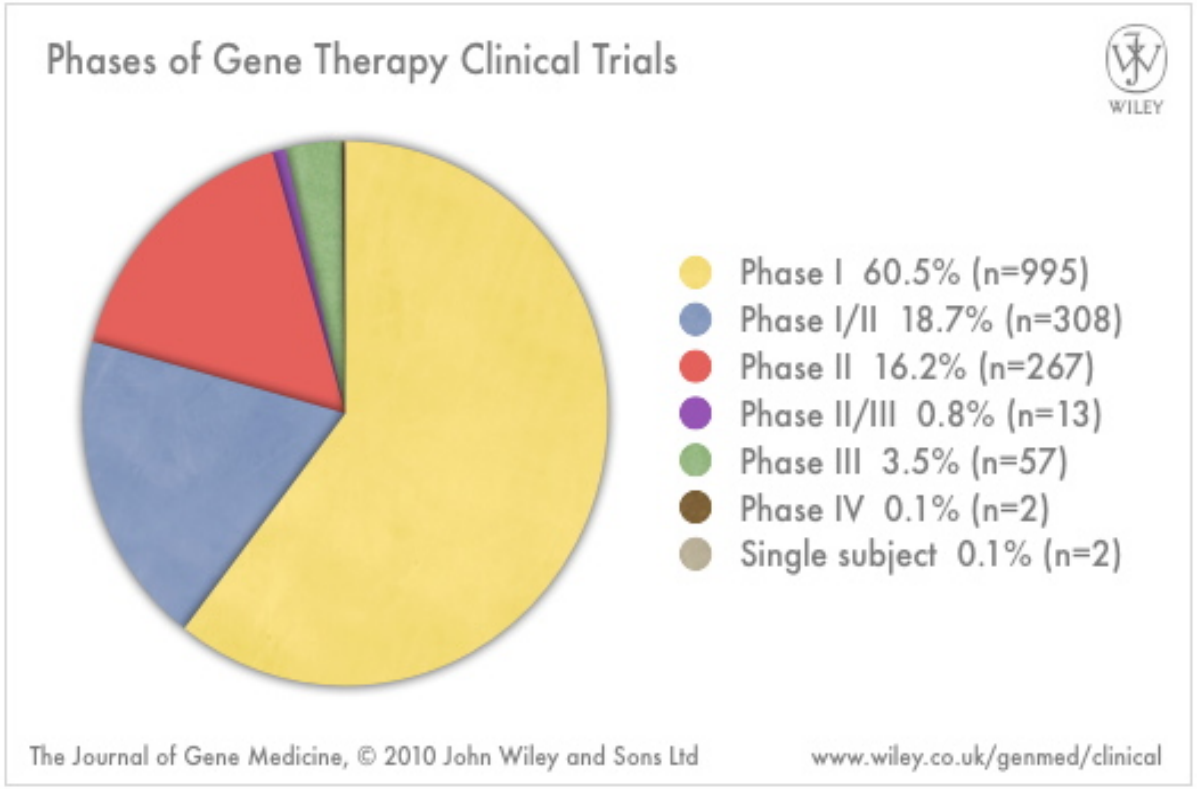
Sviluppo di una nuova terapia

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…

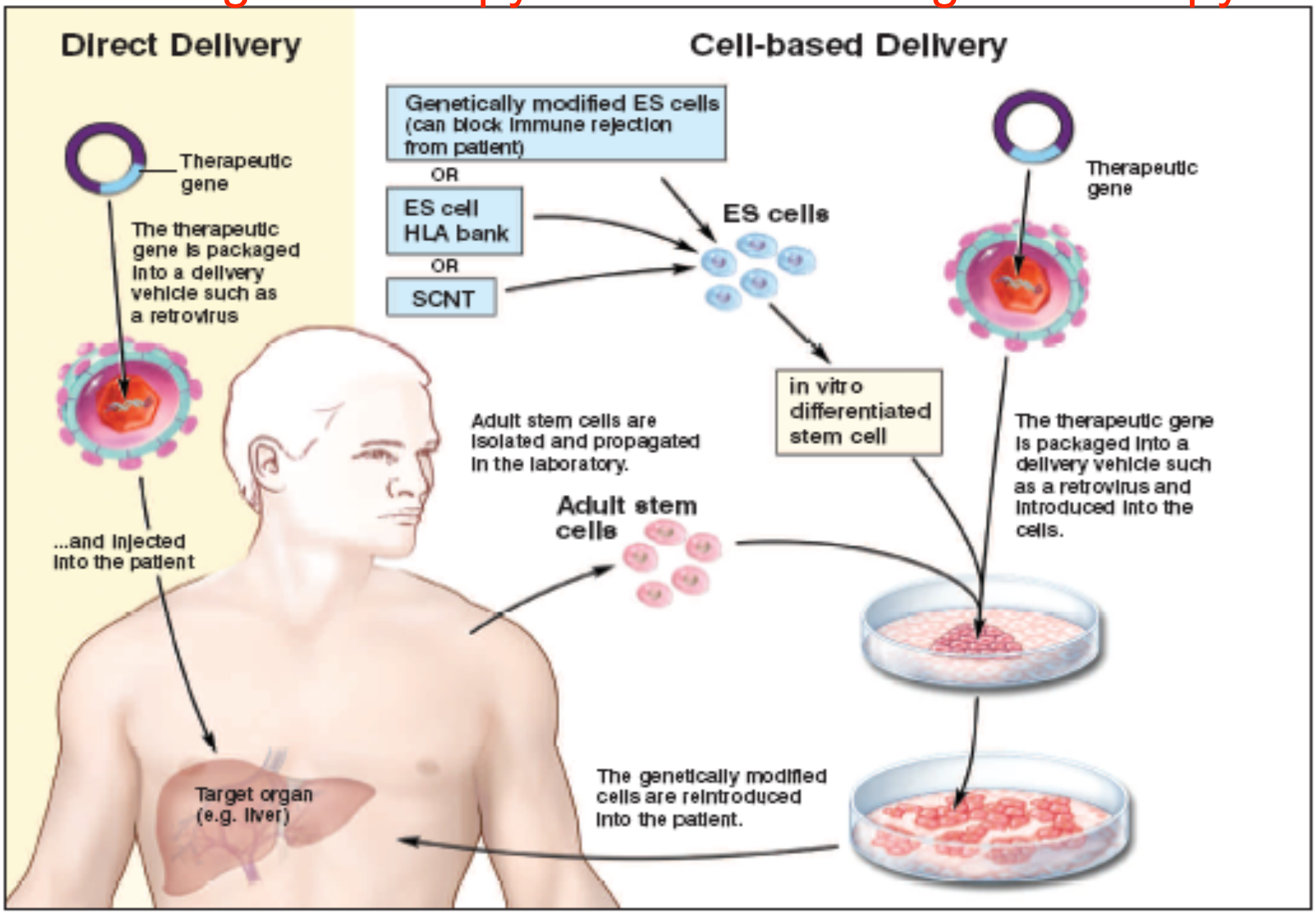
- ✓tumori localizzati; patol. dell'apparato respiratorio; tessuto cutaneo ecc…

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



Strategie di terapia genica

✓ Compensazione genica	introduzione di copie funzionali del gene difettivo o assente
✓ Riparo genico	correzione del gene difettivo
✓ Inattivazione	introduzione di RNA antisenso per inibire l'espressione genica
✓ Suicida	introduzione di "geni suicidi" che producono tossine o pro-farmaci
✓ Anti-angiogenica	interruzione del nutrimento ai tumori
✓ Anticorpale	introduzione di geni che producono anticorpi intracellulari
✓ Anti-infiammatoria	prevenzione del riconoscimento dei tessuti da parte dell'organismo
✓ Vaccinazione	introduzione di geni che inattivano agenti infettivi
✓ Terapie cellulari	trapianto di cellule geneticam modif

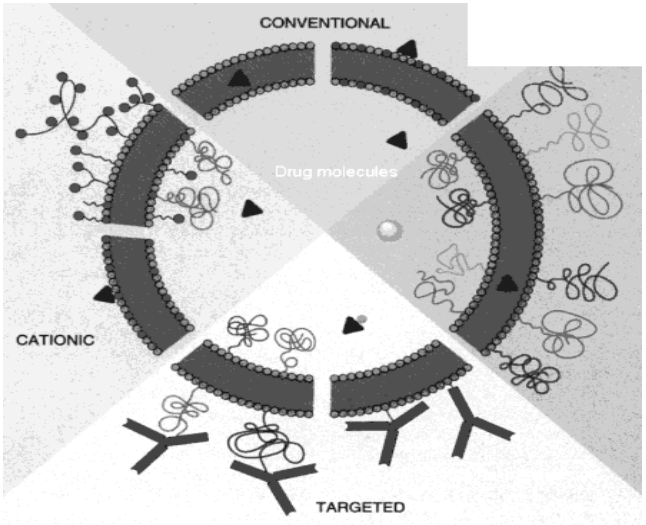
Tecniche di trasferimento genico

Lipofezione

Liposomi

Lipidi cationici

Complessi liposomi/proteine



Vettori virali

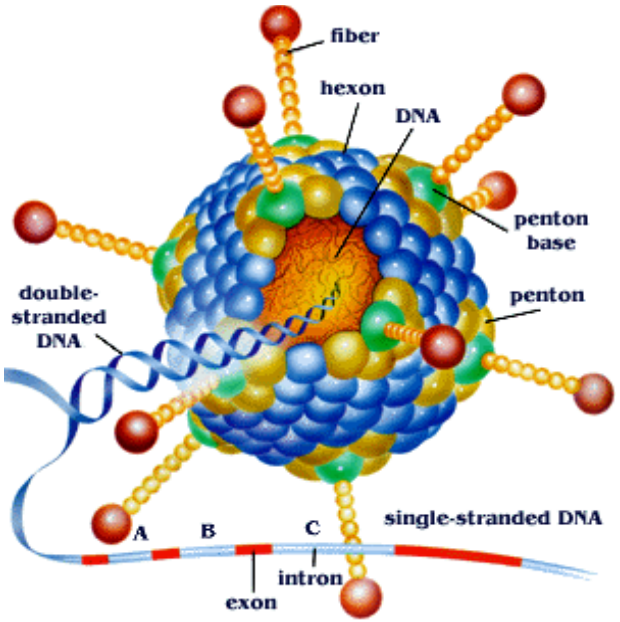
Retrovirus

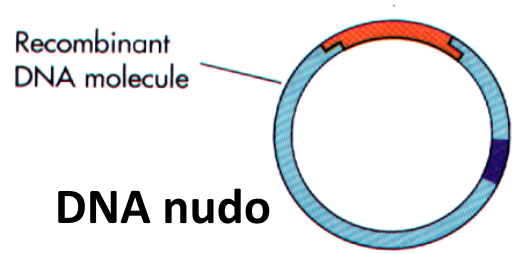
Adenoviruses

Virus adeno-associati (AAV)

Lentiviruses

Herpesviruses





Metodi fisici

Microiniezione

Elettroporazione

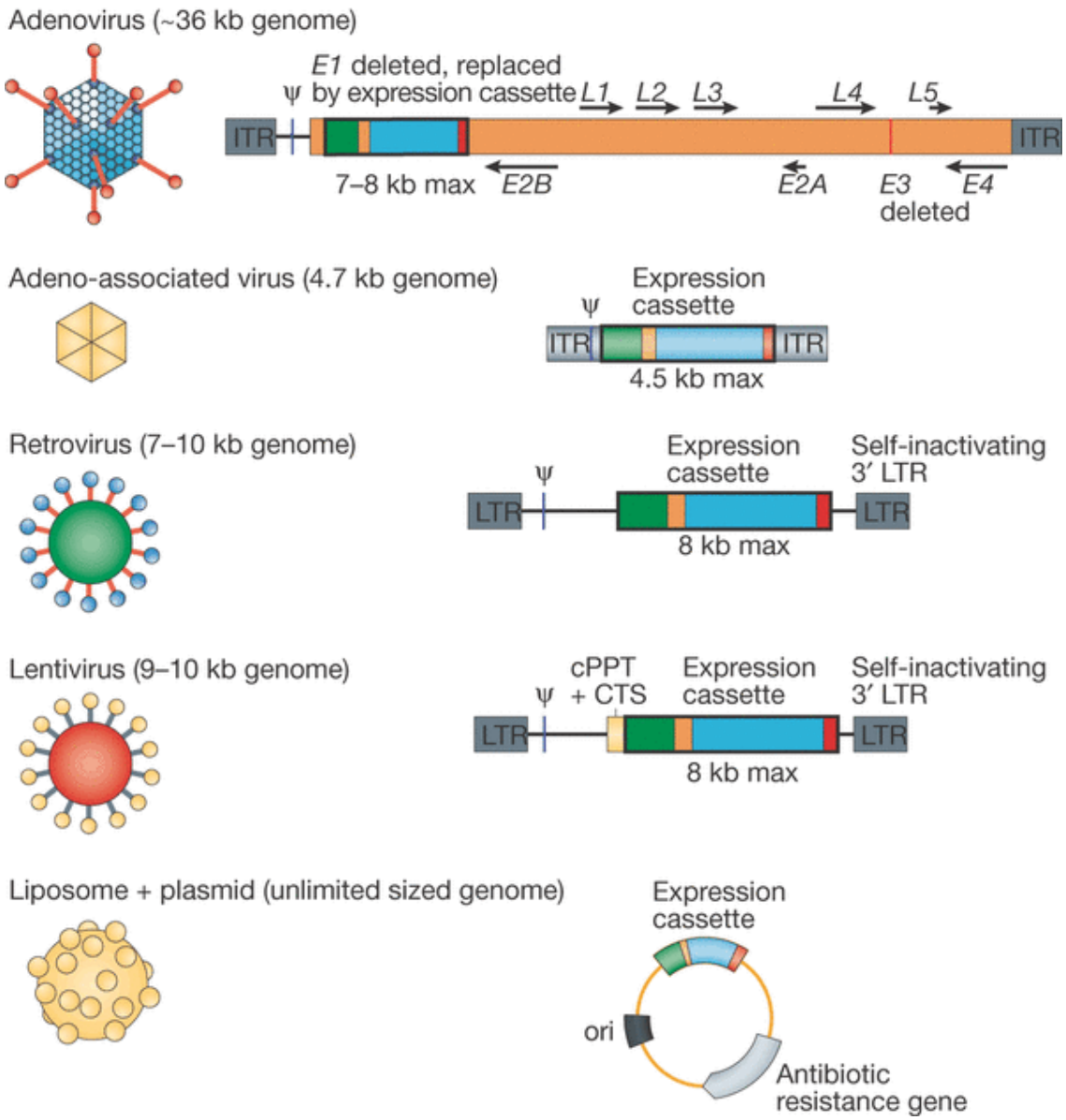
DNA complessato a nanoparticelle

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



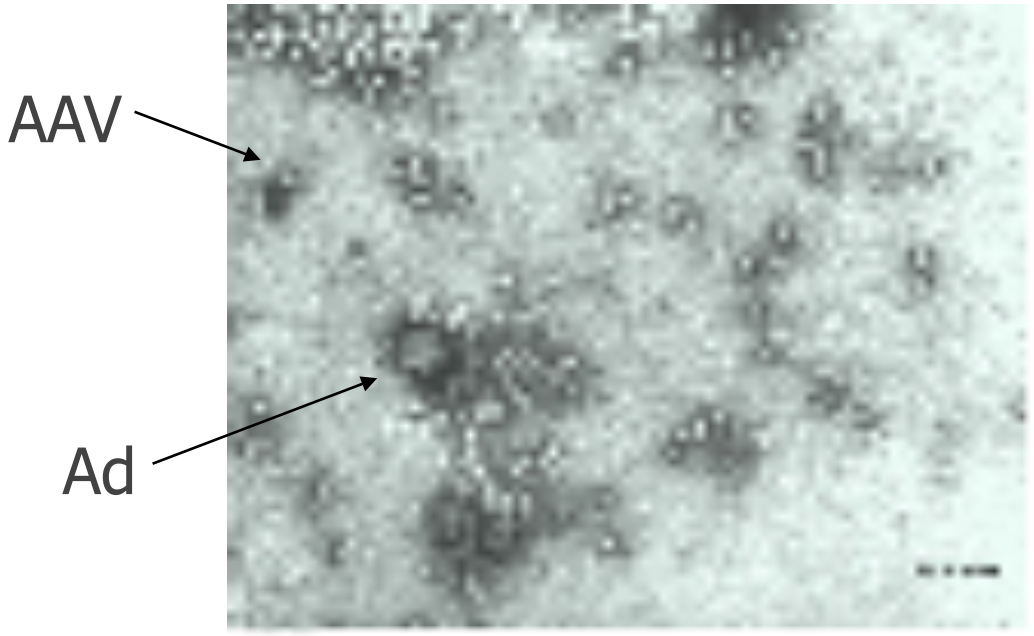
Vantaggi

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

Svantaggi

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

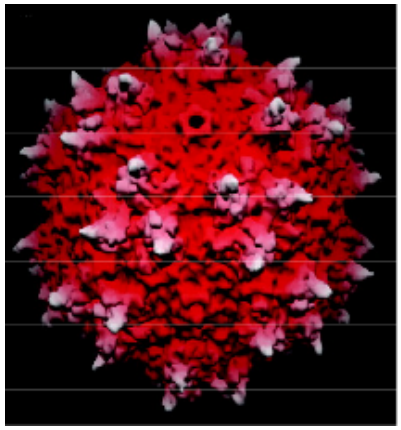
Adeno-associated virus (AAV)



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.

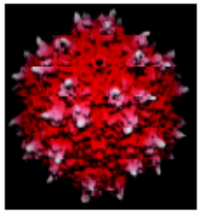
Genome
Linear, non-segmented, ssDNA ~5 kb.
AAVs package equal amounts of (+) and (-) strands.



Xie et al. 2002

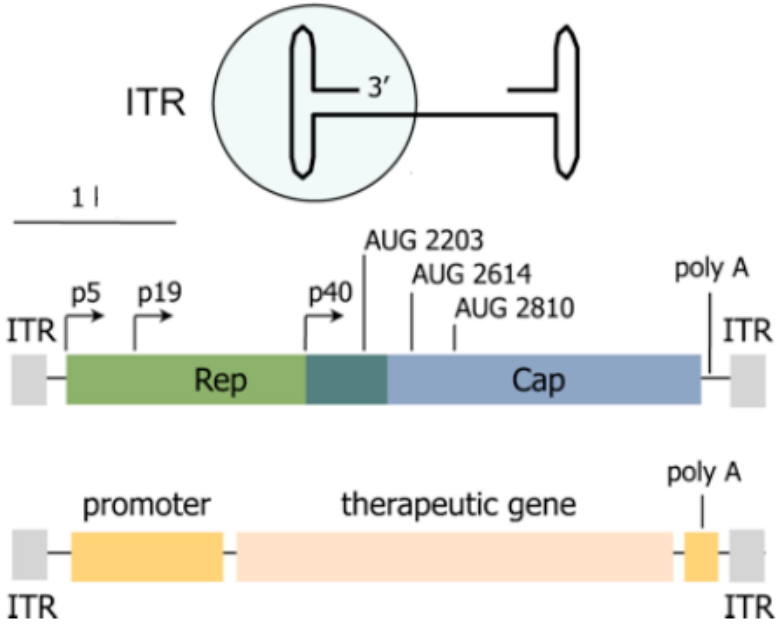
Gene transfer using Adeno-Associated Virus (AAV) vectors

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

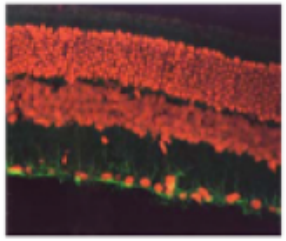


Xie et al. 2002

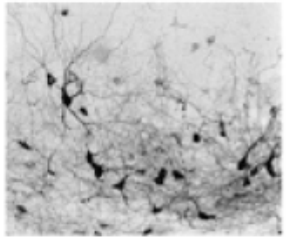
Family: Parvovirus
Genus: Dependovirus
Type: AAV 1-9
Size: 18-26 nm
Genome: ssDNA ~5 kb



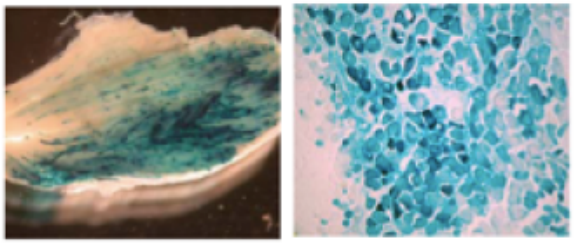
AAV vectors transduce with high efficiency post-mitotic tissues in vivo



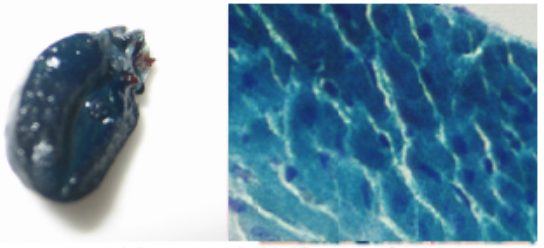
Retina, AAV2-GFP



Brain, AAV2-bcl2

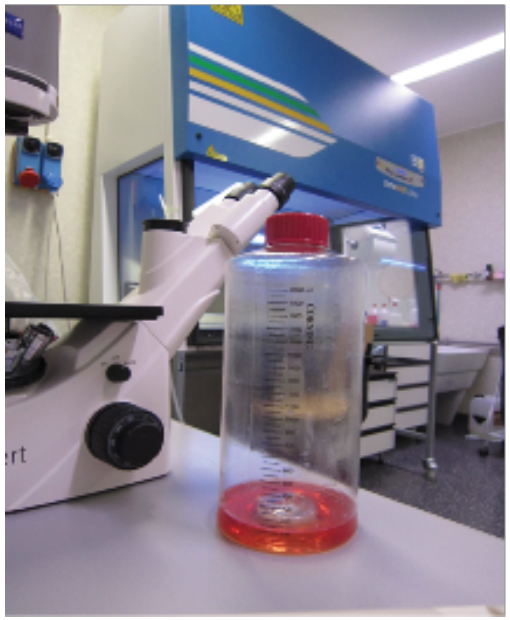


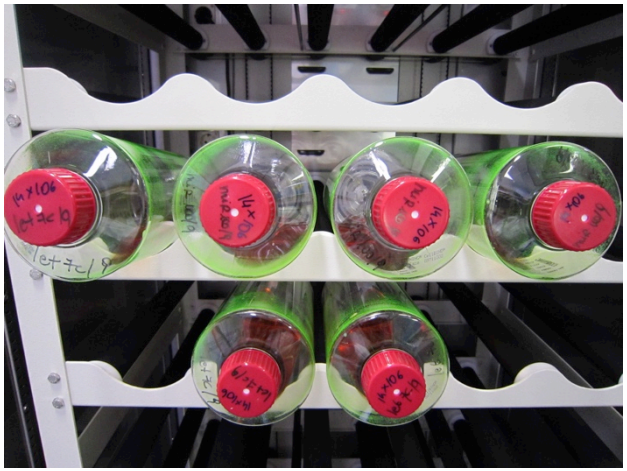
Muscle, AAV8-βgal

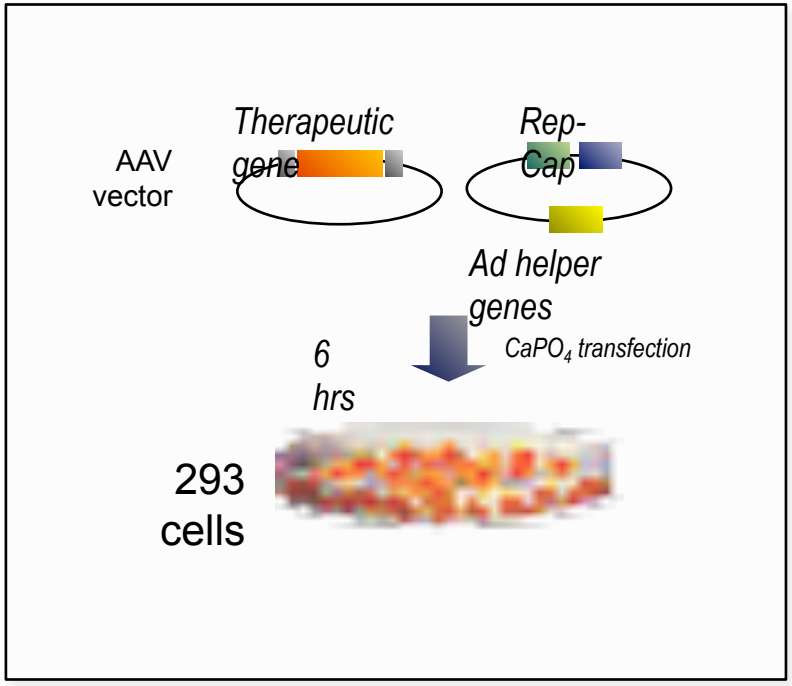


Heart, AAV9-βgal

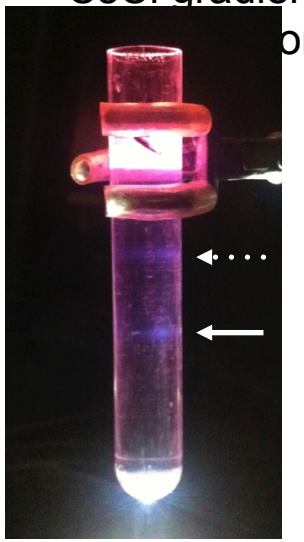
AAV Vector Unit (AVU) facility at ICGEB Trieste



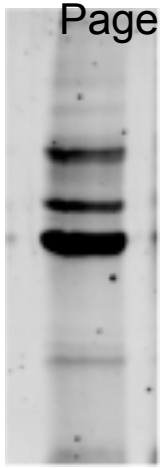




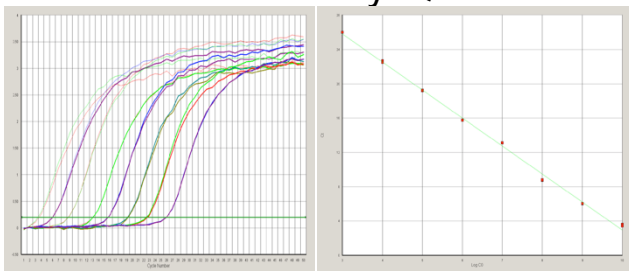
CsCl gradient



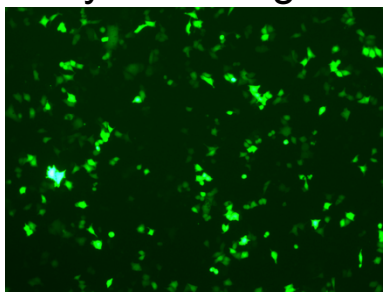
SDS Page



Titration by QC-PCR



Assay for transgene expression

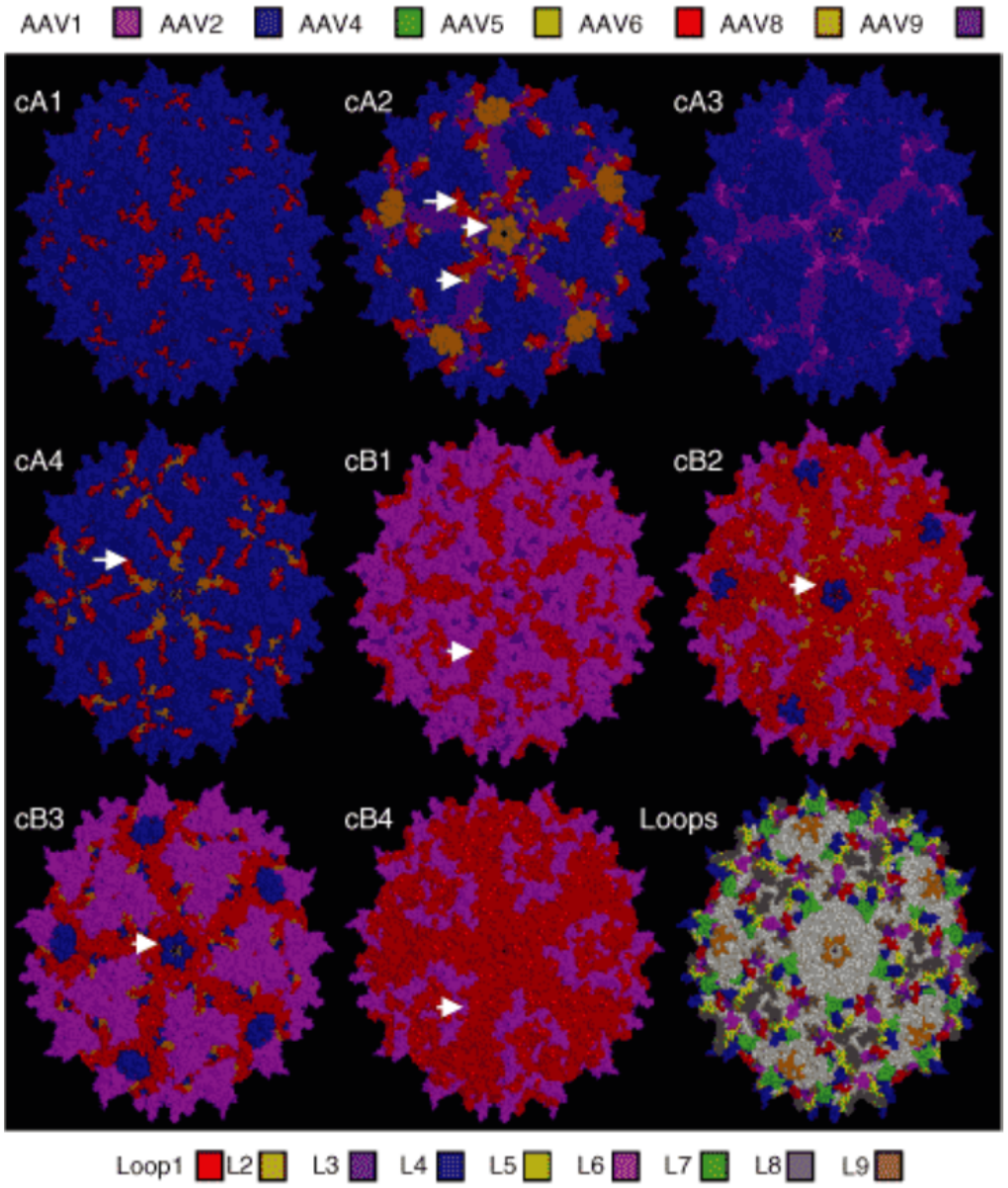


Yield: 1x10^3-1x10^4 viral particles/cell.
Physical titer: 1x10^{12} to 1x10^{13} viral genome particles per ml.
Final volume of a standard vector batch: 3 ml in phosphate buffered saline (PBS).

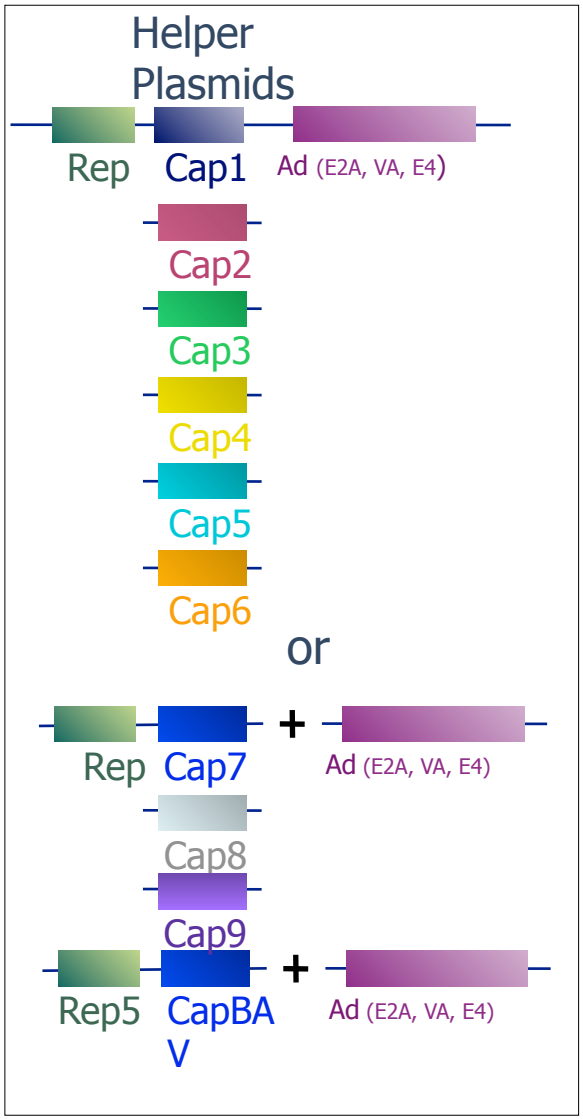
Recettori di alcuni Parvovirus

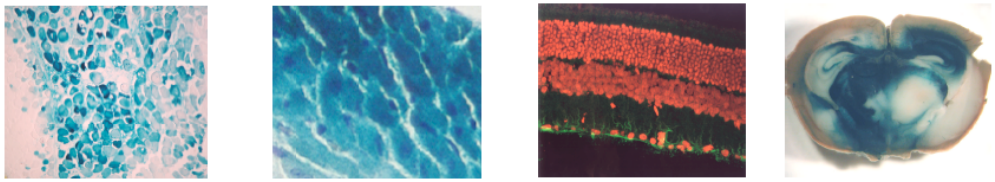
Parvovirus	Recettore
AAV1	Acido sialico (legami α2-3-N e α2-6-N)
AAV2	Proteoglicani contenenti eparan-solfati (HSPG) Corecettori: integrina $\alpha v\beta 5$, FGFR1, HGF-R
AAV3	Proteoglicani contenenti eparan-solfati (HSPG)
AAV4	Acido sialico (legami α2-3-O)
AAV5	Acido sialico (legami α2-3-O e α2-3-N) 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

New capsid variant can be created by rational engineering or DNA shuffling



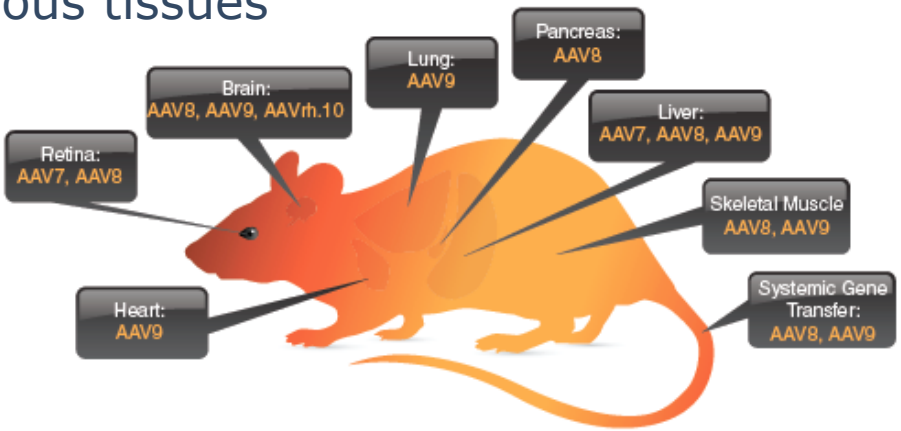
AAV efficiently transduces permissive tissues and promotes persistent transgene expression

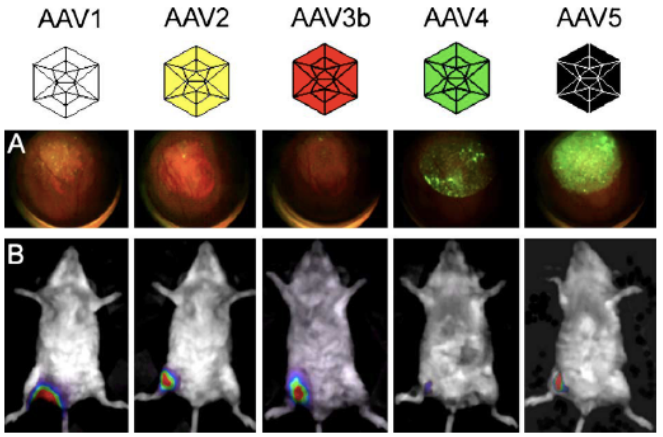


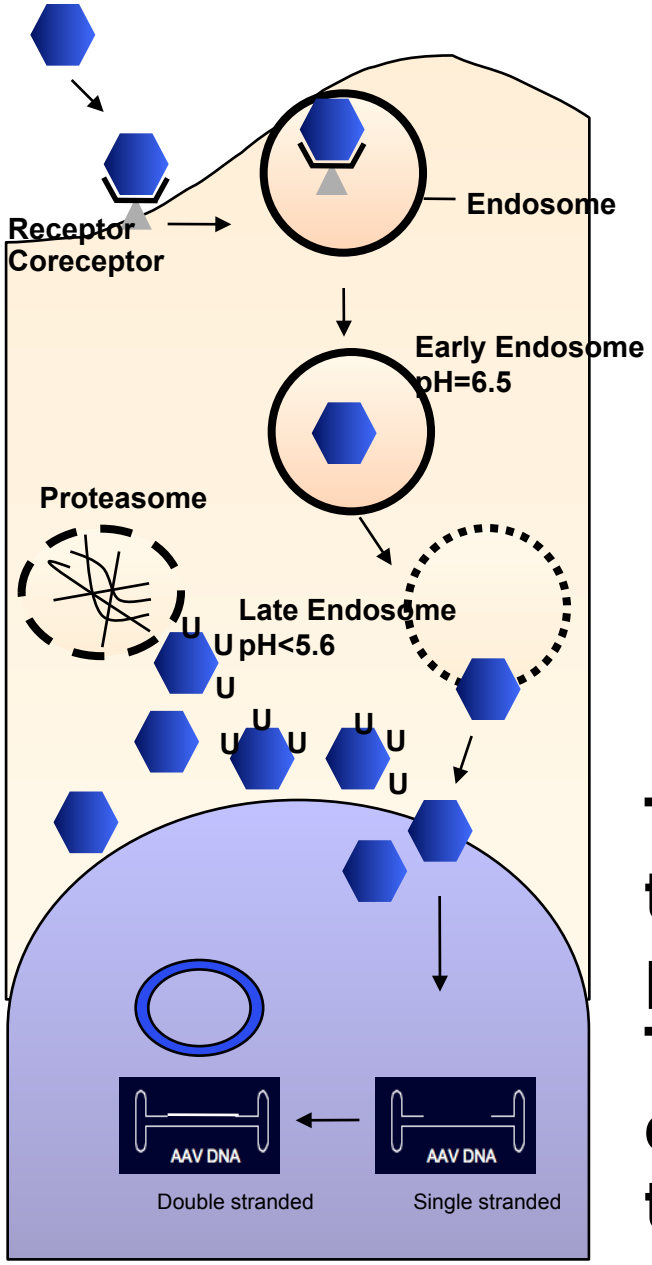


Skeletal muscle **Heart** **Retina** **Brain**

Optimal AAV serotypes for in vivo gene transfer to various tissues





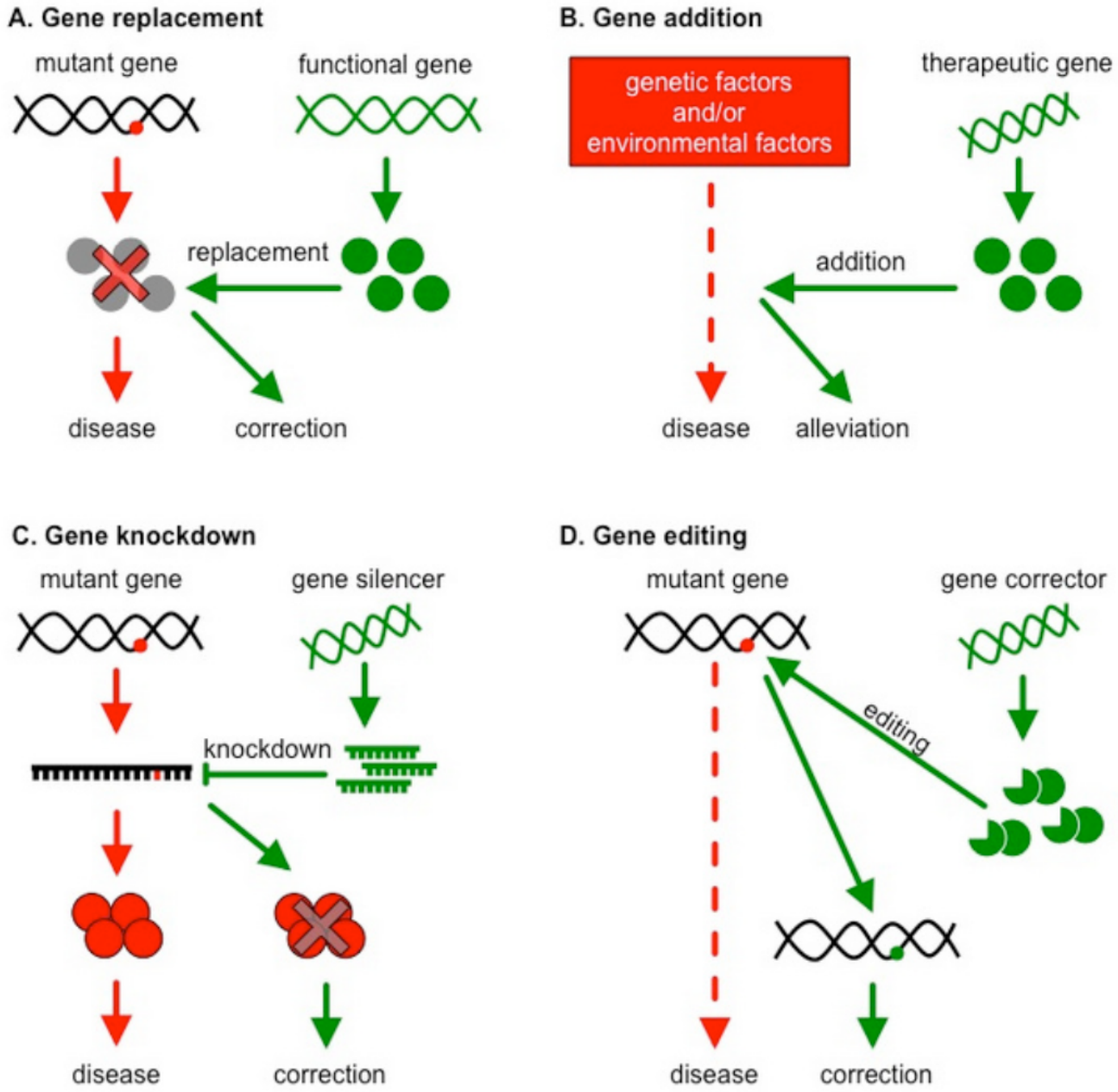


Infectious entry pathways of AAV vectors

- Processing of AAV particles in endosomes
- AAV trafficking from the cell membrane to the nucleus
- Virion uncoating in the nucleus
- Genome conversion from single stranded to double stranded DNA and stability

The events that lead to efficient transduction of AAV vectors are still poorly understood.
Tropism may depend on the ability of different cell types to support these events

Four Gene Therapy Strategies



Clinical trials using AAV vectors

Medical Condition	Disease	Gene	Route	Phase	Status	PI
Cancer	Prostate cancer	GM-CSF	Ex vivo, intradermal	I-III	4 Open/2 Completed	Gillison ML, Drake C, Corman JM, Curti B, Urba WJ
	Melanoma	B7-2, IL-12	Ex vivo, intradermal	I	1 Open	Dummer R
Cardiovascular	Heart failure	SERCA-2a	Intracoronary	I	2 Open	London B, Jessup M
Infectious diseases	HIV-1 vaccination	HIV-1 gag-pro-prt	Intramuscular	I	2 Open	Clumeck N, van Lunzen J
Monogenic diseases	Lipoprotein Lipase deficiency	Lipoprotein Lipase S447X	Intramuscular	I-II	1 Open	Stroes E
	Leber amaurosis	RPE65	Intraocular	I	2 Open	Byrne B, Jacobson S, Maguirre A
	Hemophilia B	FIX	Intramuscular/Liver	I	2 Completed/1 Under review	Manno C, Glader B, Nienhuis A
	Cystic fibrosis	CFTR	Intranasal, Intrapulmonary	I-II	6 Completed	Gardner P, Aitken M
	Limb girdle distrophy	Sarcoglycan	Intramuscular	I	8 Open/2 Completed	Mendell J
	Duchenne muscular Dystrophy	Minidystrophin	Intramuscular	I	1 Open	Mendell J
	alpha1-antitrypsin deficiency	AAT	Intramuscular	I	2 Open	Flotte T
Neurological diseases	Parkinson disease	GAD, AADC-2, Neurturin	Intracranial	I-II	2 Open/ 1 Completed	Marks W, Verhagen L
	Alzheimer disease	NGF	Intracranial	I-II	1 Completed	Bennett D
	Intractable epilepsy	NPY	Intracranial	I	1 Open	During M
	Canavan disease	Aspartocyclase	Intracranial	I	1 Open/ 2 Completed	Seashore MR, Freeze A, Leone P
	Late infantile neuronal ceroid lipofuscinosis	Tripeptydil peptidase	Intracranial	I	1 Open	Crystal R
	Amyotrophic Lateral Sclerosis	EAAT2	Intracranial	I	1 Under review	During M
Others	Rheumatoid arthritis	TNFR:Fc	Intra-articular	I	2 Open	Mease P
Gene marking		hpAP	Intranasal/ Intrabronchial	I	2 Open	Aitken M

Peculiarities of the eye as a target for gene therapy

The eye is a site of immune-priviledge

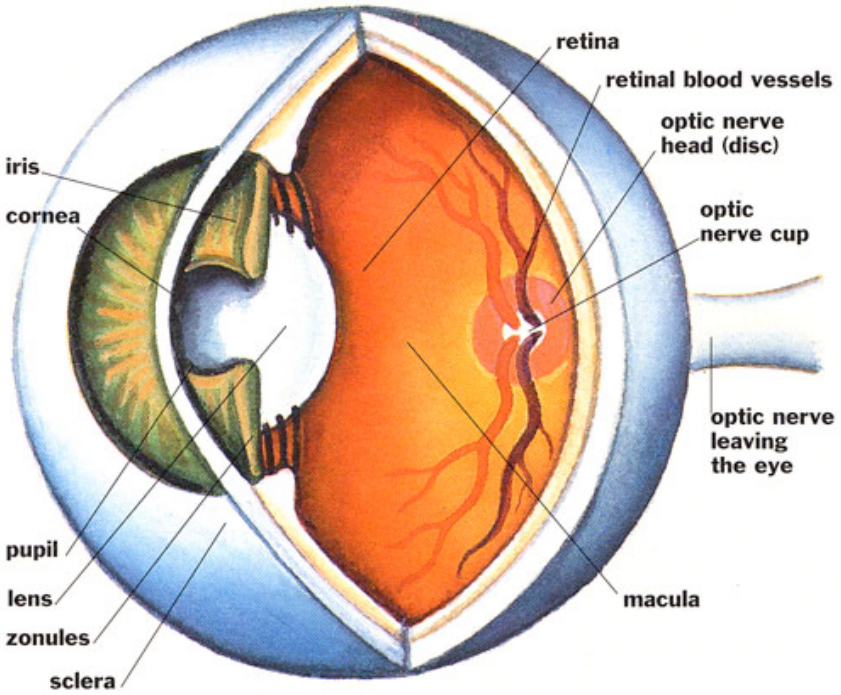
Most cells in the post-natal eye are terminally differentiated and prone to degenerative processes

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

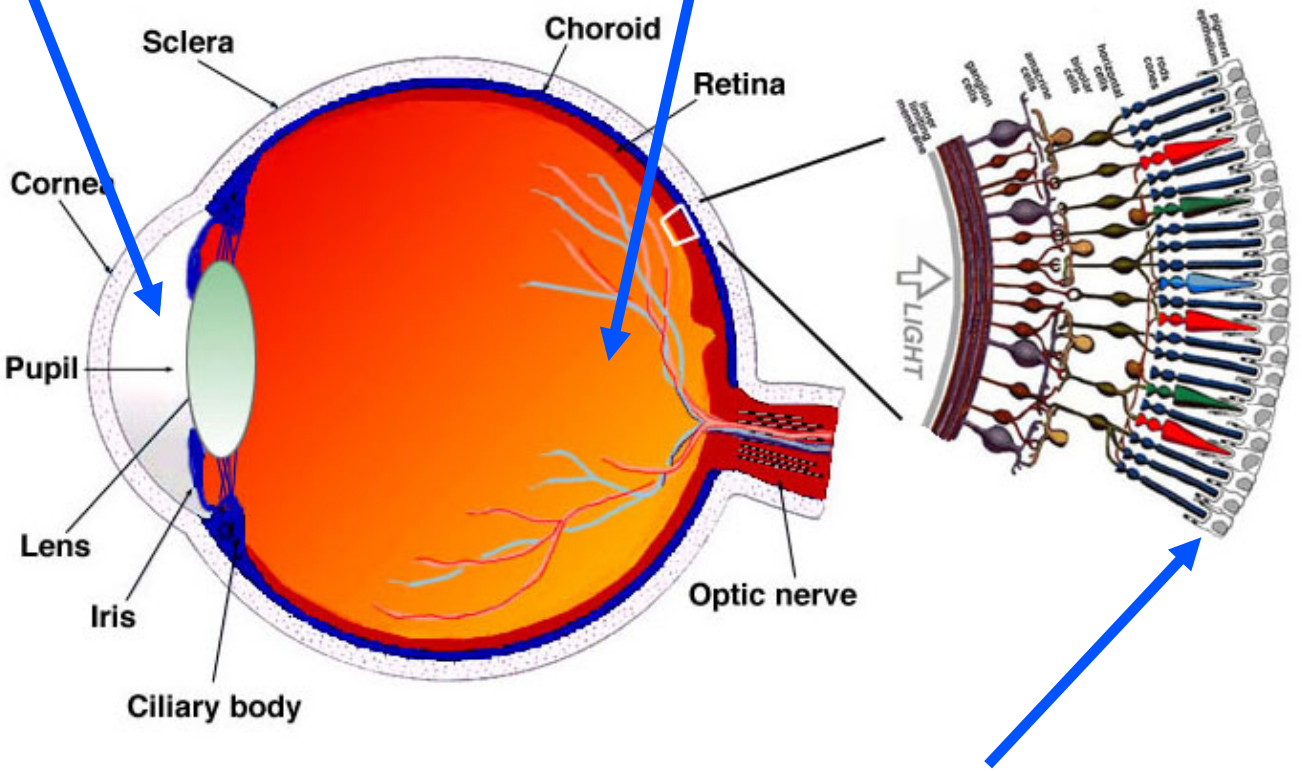
The results of the first clinical trials for ocular cancer and angiogenic disease have now been reported. One trial of gene replacement therapy for inherited retinal degeneration commenced recently and further such trials are expected to begin imminently

There are many animal models available



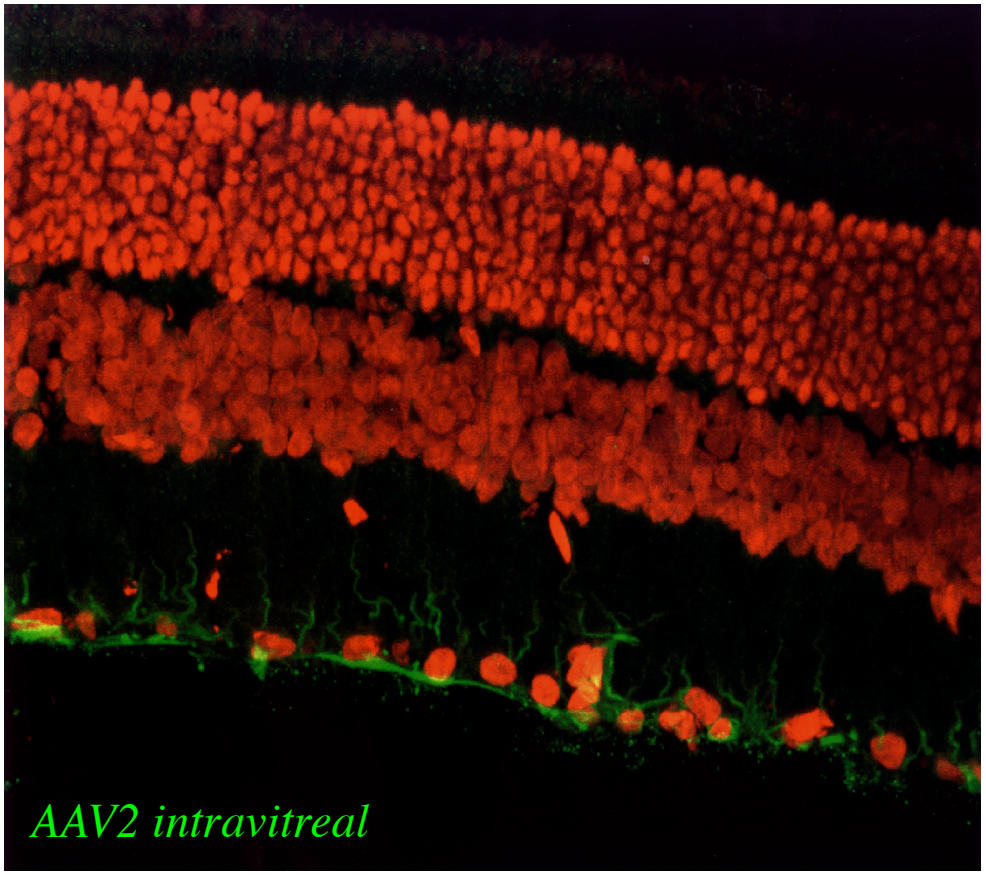
Into the anterior chamber - for corneal disease

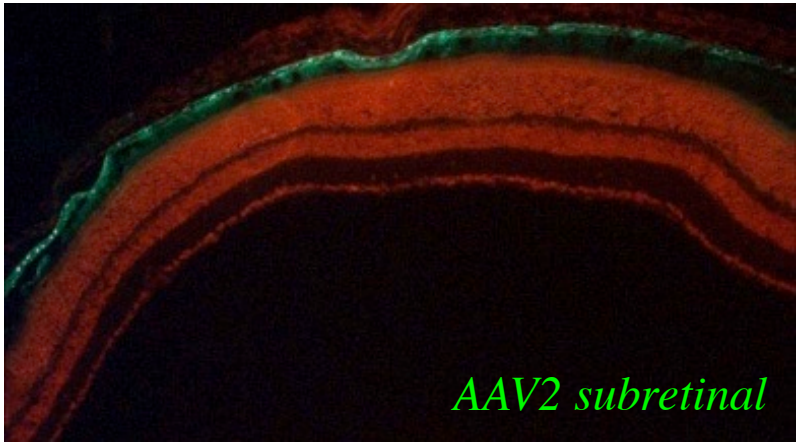
Intravitreal - anti-apoptotic or neurotrophic genes to protect RGC death in glaucoma

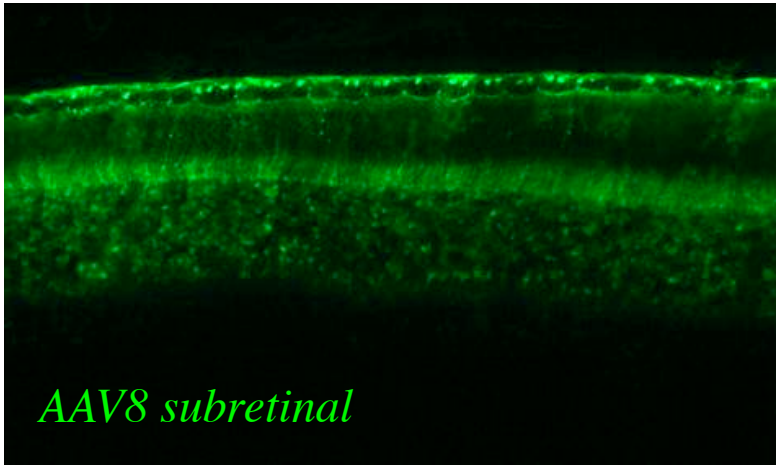


Sub-retinal (between photoreceptors and RPE) - for inherited retinal disorders, retinoblastoma and retinal neovascularization

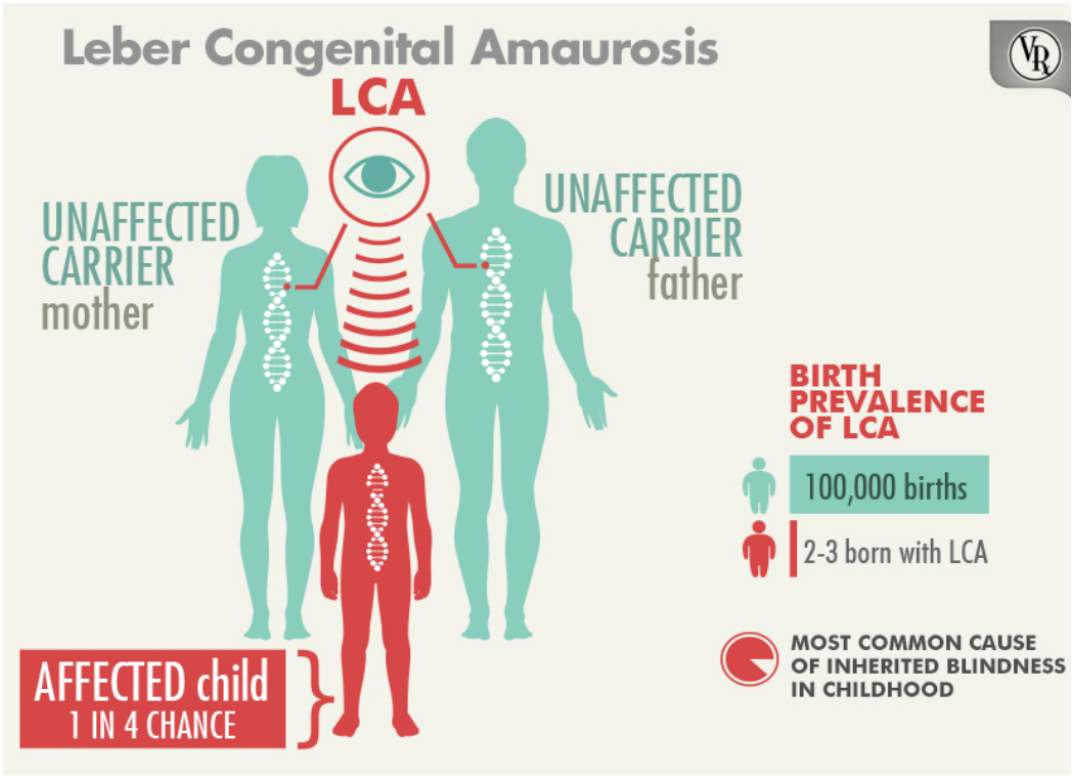
AAV is the only vector to efficiently transduce both RPE and photoreceptors

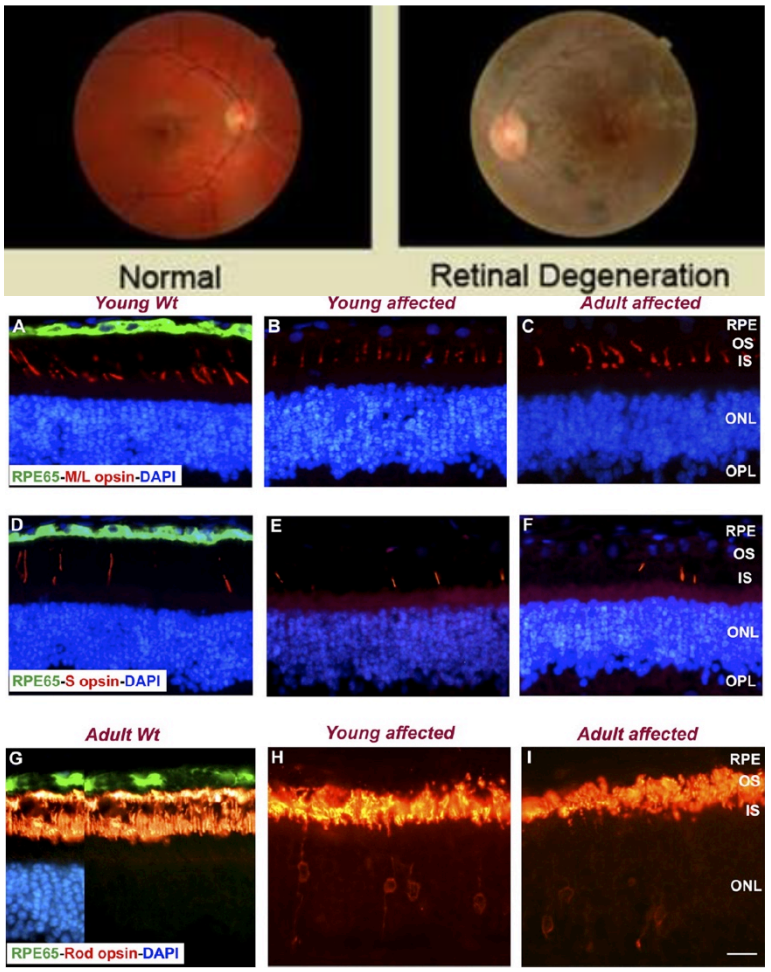






Leber Congenital Amaurosis





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

Leber Congenital Amaurosis due to RPE65 Mutations

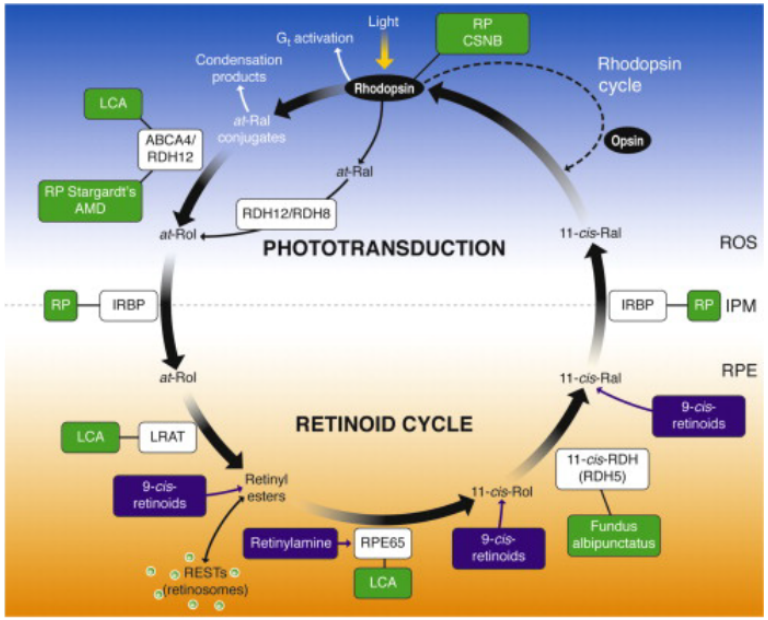
Restoration of RPE65 in RPE cells is predicted to result in production of 11-cis-retinal and in reactivation of PR function
Excellent and consistent preclinical results obtained in small and large animal models

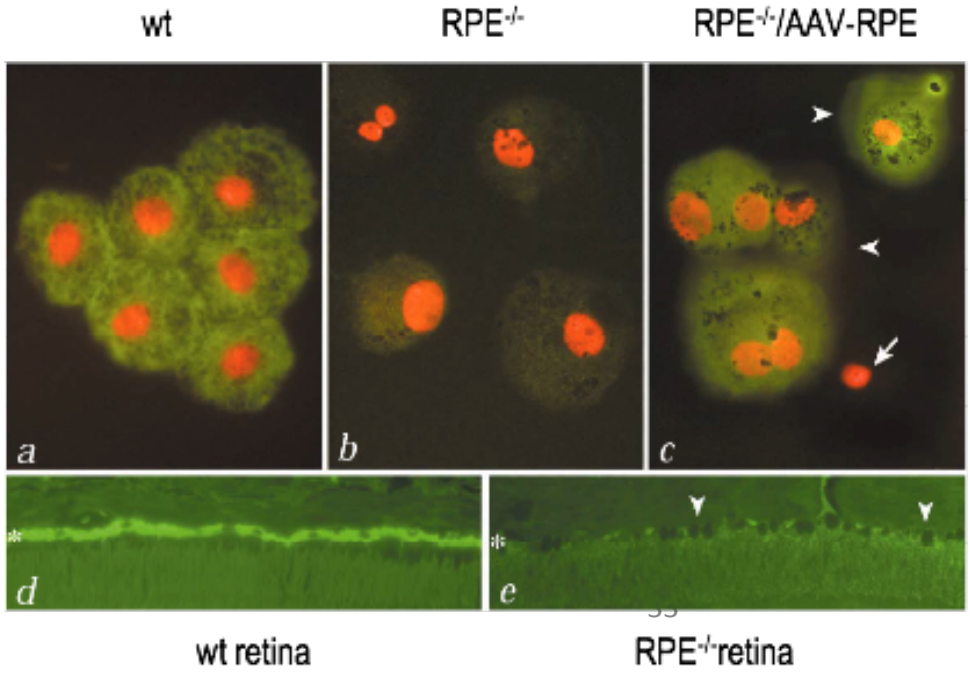
RPE65-/- dogs bear a homozygous 4bp deletion resulting in a frameshift and a premature stop codon

letter

Gene therapy restores vision in a canine model of childhood blindness

Gregory M. Acland[1], Gustavo D. Aguirre[1], Jharna Ray[1], Qi Zhang[1], Tomas S. Aleman[2], Artur V. Cideciyan[2], Susan E. Pearce-Kelling[1], Vibha Anand[2], Yong Zeng[2], Albert M. Maguire[2], Samuel G. Jacobson[2], William W. Hauswirth[3] & Jean Bennett[2]





HUMAN GENE THERAPY 17:845–858 (August 2006)

Safety in Nonhuman Primates of Ocular AAV2-*RPE65*, a Candidate Treatment for Blindness in Leber Congenital Amaurosis

SAMUEL G. JACOBSON,[1] SANFORD L. BOYE,[2] TOMAS S. ALEMAN,[1] THOMAS J. CONLON,[3] CAROLINE J. ZEISS,[4] ALEJANDRO J. ROMAN,[1] ARTUR V. CIDECIYAN,[1] SHARON B. SCHWARTZ,[1] ANDRAS M. KOMAROMY,[5] MICHELLE DOOBRAJH,[1] ANDY Y. CHEUNG,[1] ALEXANDER SUMAROKA,[1] SUSAN E. PEARCE-KELLING,[6] GUSTAVO D. AGUIRRE,[5] SHALESH KAUSHAL,[2] ALBERT M. MAGUIRE,[1] TERENCE R. FLOTTE,[3] and WILLIAM W. HAUSWIRTH[2,3]

- No systemic toxicity, only modest local inflammation
- No photoreceptor abnormalities after AAV delivery

BRIEF REPORT

Safety and Efficacy of Gene Transfer for Leber's Congenital Amaurosis

Albert M. Maguire, M.D., Francesca Simonelli, M.D., Eric A. Pierce, M.D., Ph.D., Edward N. Pugh, Jr., Ph.D., Federico Mingozzi, Ph.D., Jeannette Bennicelli, Ph.D., Sandro Banfi, M.D., Kathleen A. Marshall, C.O.T., Francesco Testa, M.D., Enrico M. Surace, D.V.M., Settimio Rossi, M.D., Arkady Lyubarsky, Ph.D., Valder R. Arruda, M.D., Barbara Konkle, M.D., Edwin Stone, M.D., Ph.D., Junwei Sun, M.S., Jonathan Jacobs, Ph.D., Lou Dell'Osso, Ph.D., Richard Hertle, M.D., Jian-xing Ma, M.D., Ph.D., T. Michael Redmond, Ph.D., Xiaosong Zhu, M.D., Bernd Hauck, Ph.D., Olga Zelenaia, Ph.D., Kenneth S. Shindler, M.D., Ph.D., Maureen G. Maguire, Ph.D., J. Fraser Wright, Ph.D., Nicholas J. Volpe, M.D., Jennifer Wellman McDonnell, M.S., Alberto Auricchio, M.D., Katherine A. High, M.D., and Jean Bennett, M.D., Ph.D.

BRIEF REPORT

Effect of Gene Therapy on Visual Function in Leber's Congenital Amaurosis

James W.B. Bainbridge, Ph.D., F.R.C.Ophth., Alexander J. Smith, Ph.D., Susie S. Barker, Ph.D., Scott Robbie, M.R.C.Ophth., Robert Henderson, M.R.C.Ophth., Kamaljit Balaggan, M.R.C.Ophth., Ananth Viswanathan, M.D., F.R.C.Ophth., Graham E. Holder, Ph.D., Andrew Stockman, Ph.D., Nick Tyler, Ph.D., Simon Petersen-Jones, Ph.D., Shomi S. Bhattacharya, Ph.D., Adrian J. Thrasher, Ph.D., M.R.C.P., F.R.C.P., Fred W. Fitzke, Ph.D., Barrie J. Carter, Ph.D., Gary S. Rubin, Ph.D., Anthony T. Moore, F.R.C.Ophth., and Robin R. Ali, Ph.D.

- 3 patients in each study
- The procedure appear safe and might be beneficial - longer follow-up needed

The NEW ENGLAND JOURNAL of MEDICINE MAY 22, 2008

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**

7 dicembre 2020

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

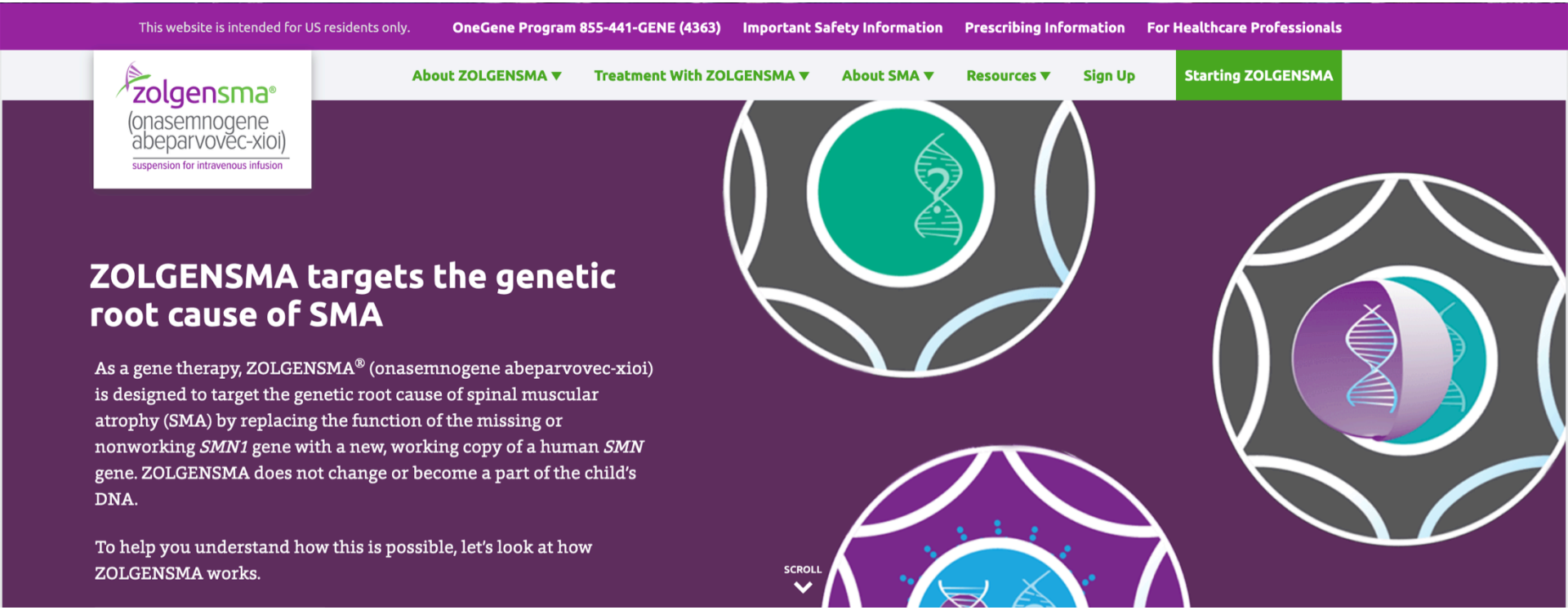
Rottamazione, «saldo e stralcio»: nuove scadenze per 1,2 milioni di italiani

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



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.

***SMN1* gene missing or nonworking**

A targeted approach

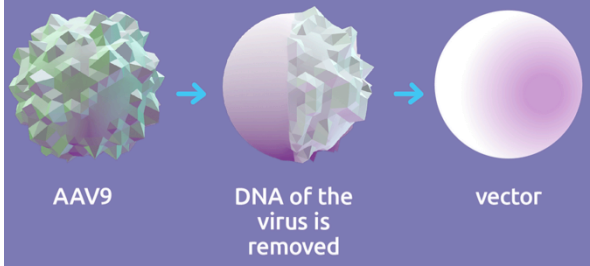
ZOLGENSMA targets the genetic root cause of SMA by replacing the function of the missing or nonworking gene, called the *SMN1* gene. This gene is critical to making SMN protein.

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).