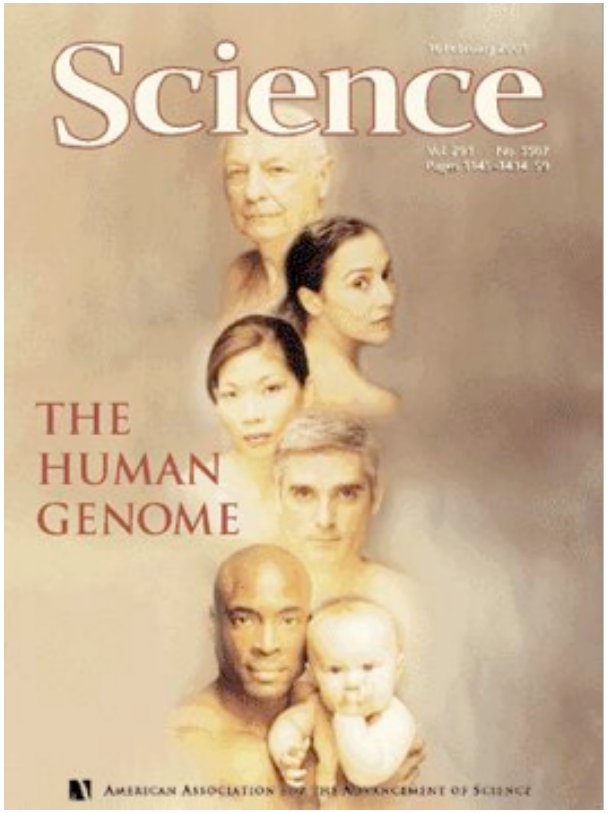
Ingegneria genetica e medicina: paure e perplessità



Che 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

Che 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.

Biomedico: produzione di farmaci ricombinanti

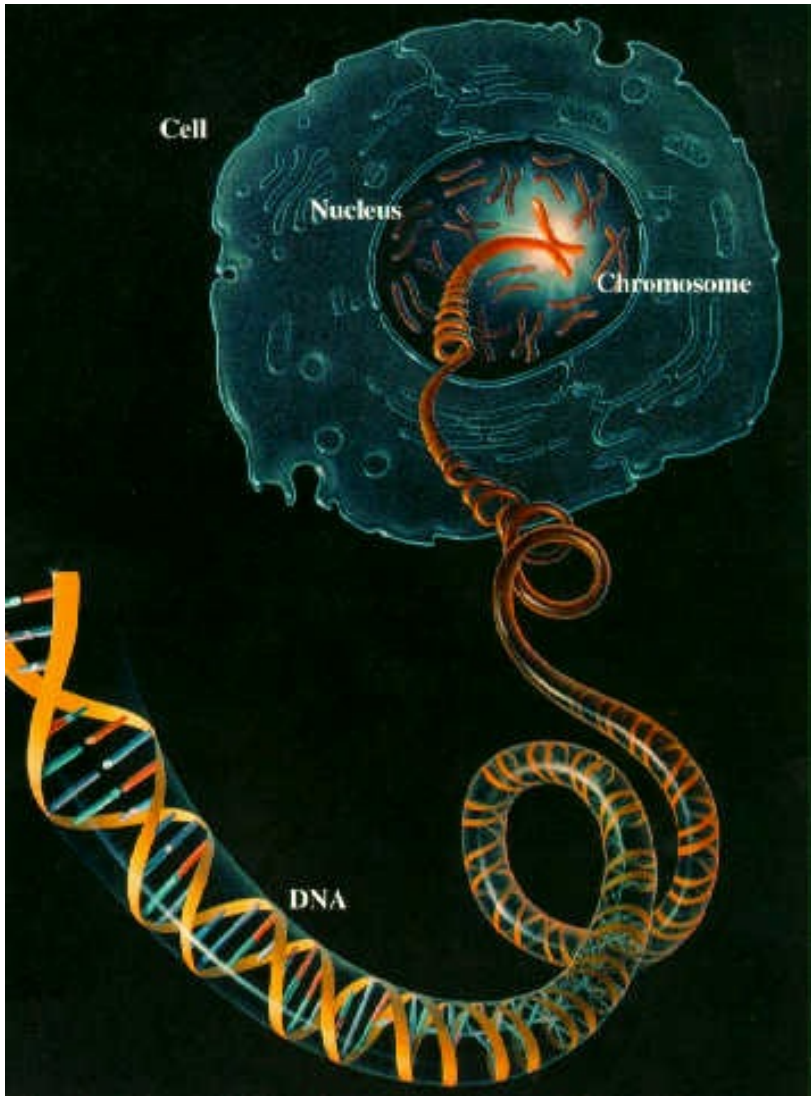
Agricolo: cereali resistenti agli erbicidi

L’ingegneria genetica applicata all’uomo:

la terapia genica

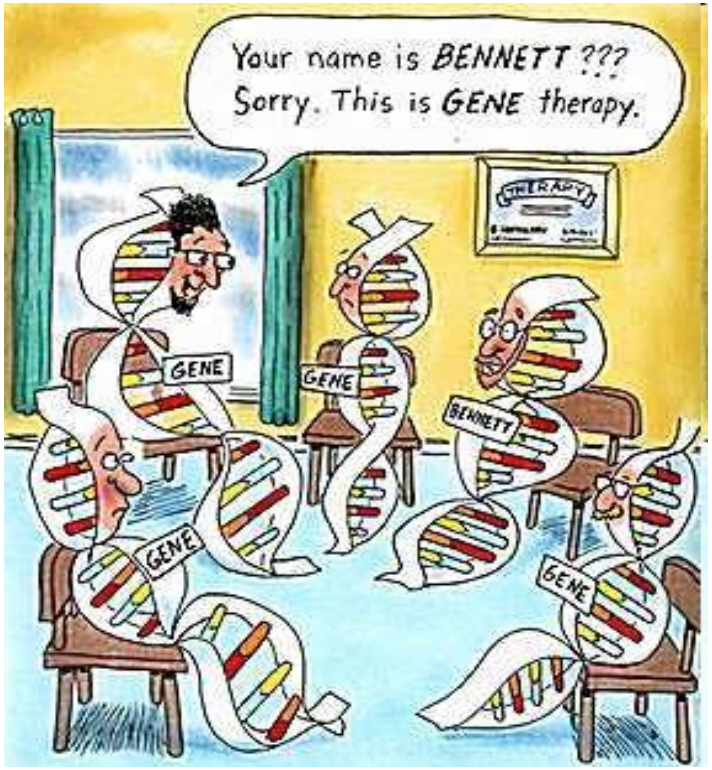
TERAPIA GENICA

- 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
- Morbidità 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 Terapia Genica

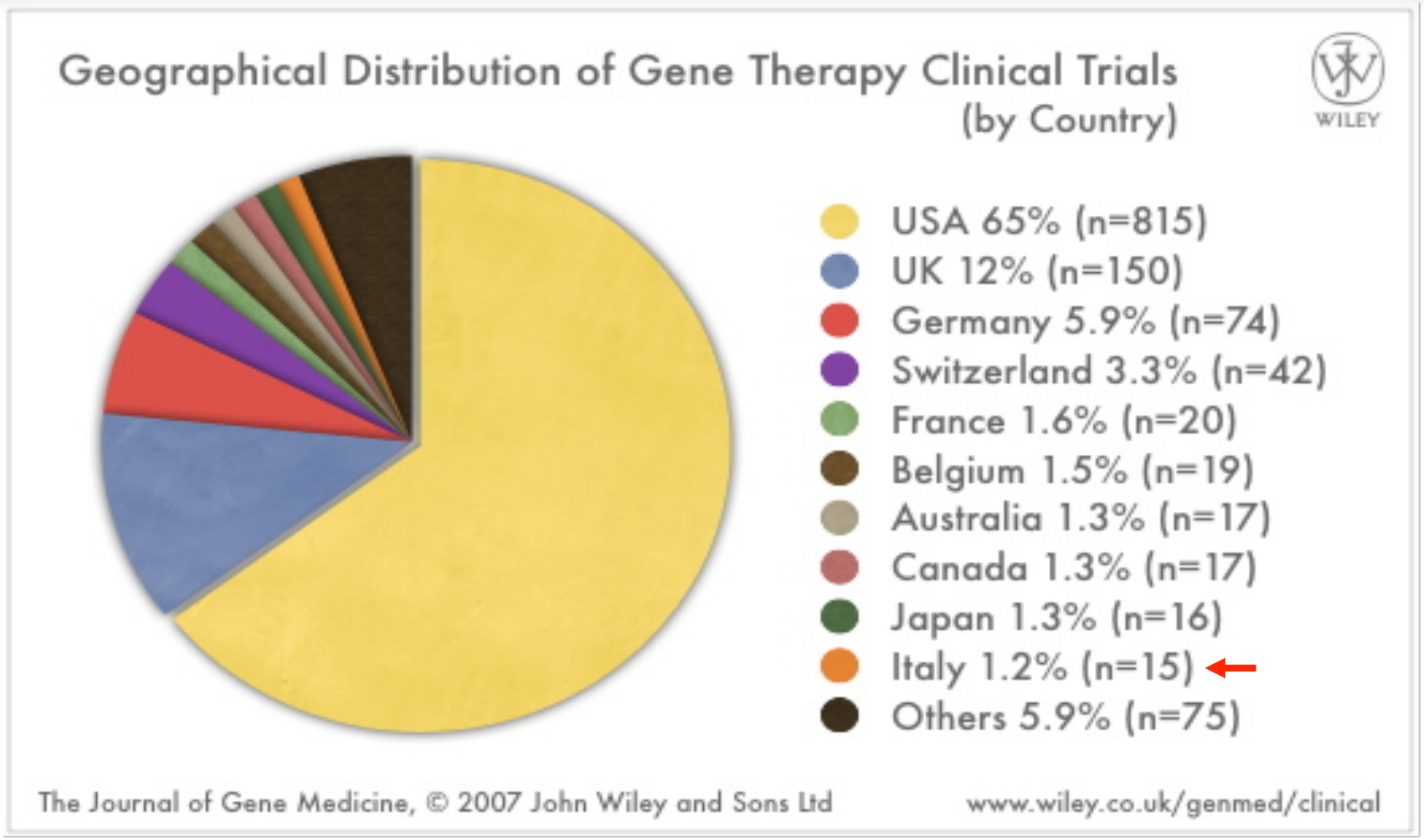
Disease	Defect	Incidence	Target Cells
Severe combined immunodeficiency (SCID)	Adenosine deaminase (ADA) in 25% of SCID patients	Rare	Bone-marrow cells or T lymphocytes
Hemophilia A	Factor VII deficiency	1:10,000 males	Liver, muscle, fibroblasts or bone marrow cells
Hemophilia B	Factor IX deficiency	1:30,000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

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	

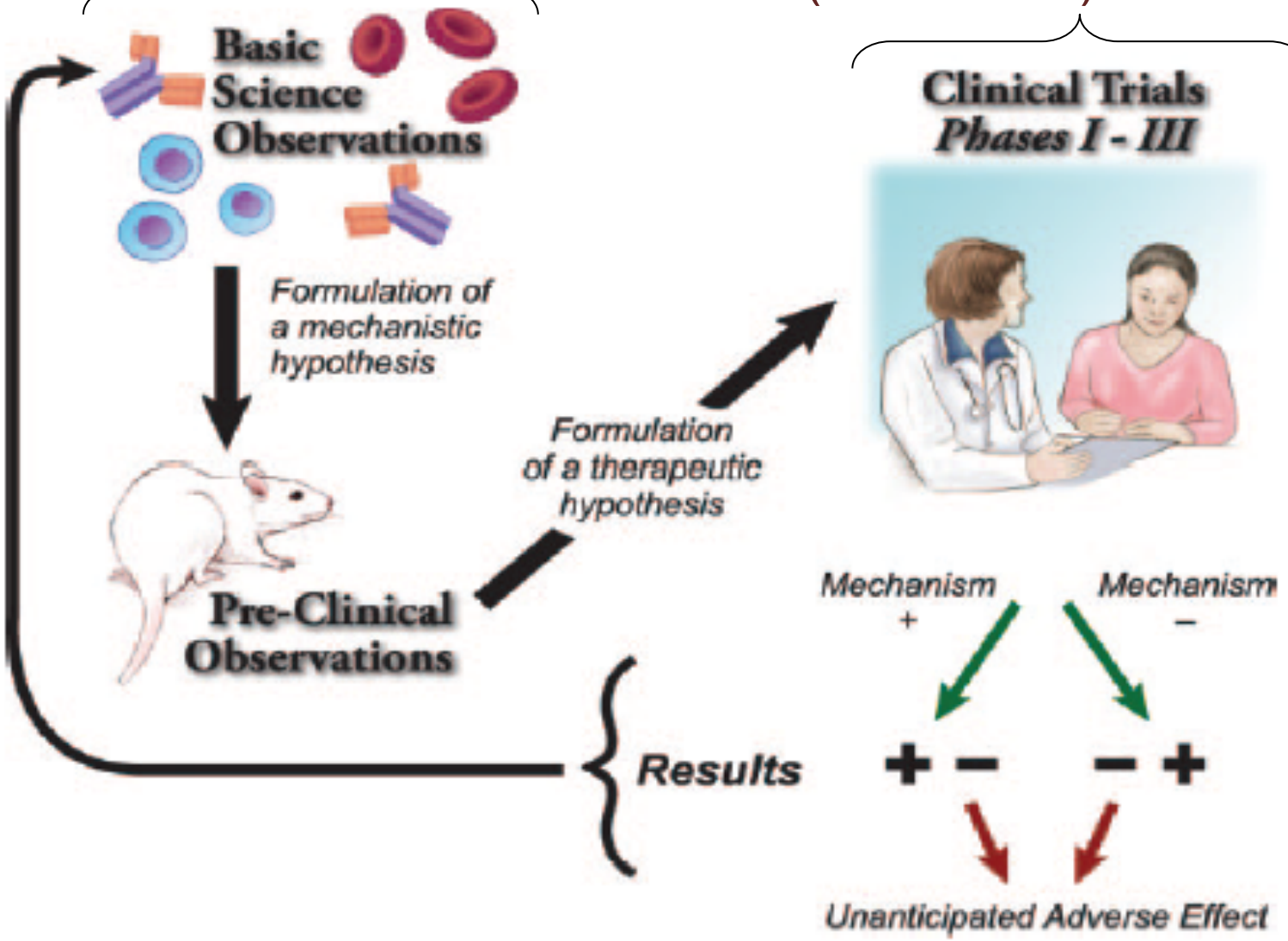
Chi investe in terapia genica?



Sviluppo di una nuova terapia

Ricerca pre-clinica

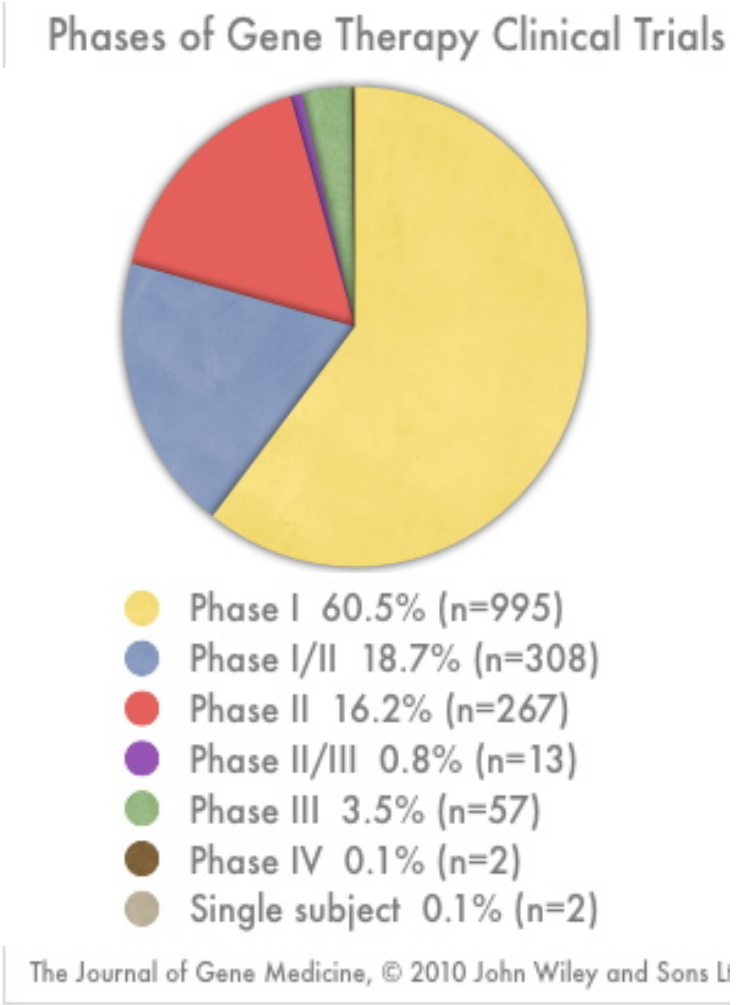
Sperimentazione clinica (trial clinico)



Trials clinici in terapia genica

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

- Fase I: si risponde al quesito: *quanto farmaco può essere somministrato senza causare effetti avversi gravi?*
- Fase II: si *valuta il meccanismo di azione del farmaco, e si continua 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, mira a trovare ogni evento avverso raro o 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 vivo

il transgene viene somministrato per via sistemica

- ✓ cellule e tessuti poco accessibili;
- ✓ scarsa efficienza di trasduzione, barriere fisiche

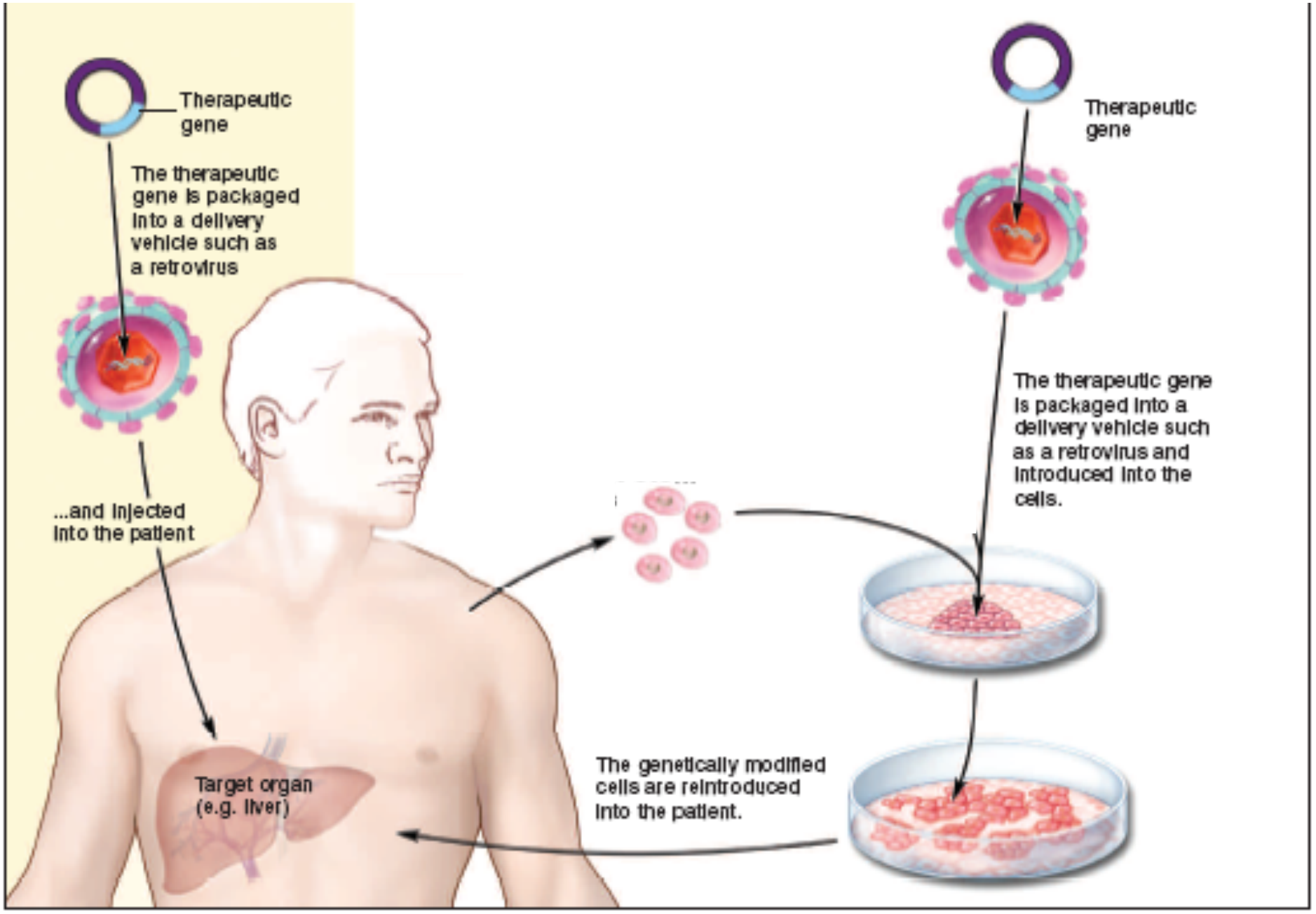
in situ

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 gene therapy

Ex vivo gene therapy



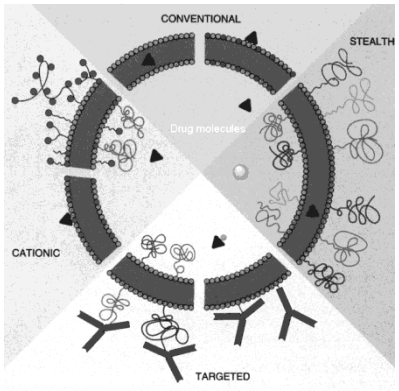
Strategie di terapia genica

- ✓ Compensazione genica
- ✓ Riparo genico
- ✓ Inattivazione
- ✓ Suicida
- ✓ Anti-angiogenica
- ✓ Anticorpale
- ✓ Anti-infiammatoria
- ✓ Vaccinazione
- ✓ Terapie cellulari

Come raggiungere il nucleo della cellula?

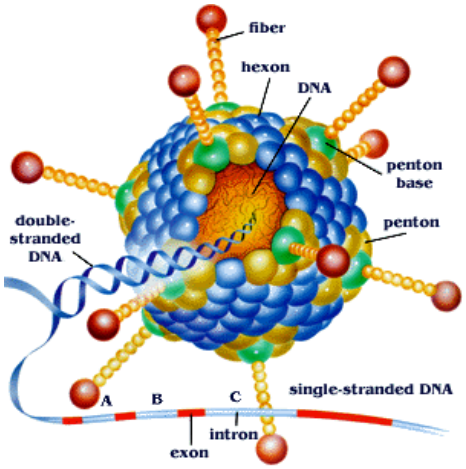
Tecniche di trasferimento genico

Lipofezione



Liposomi
Lipidi cationici
Complessi liposomi/proteine
SNALPS

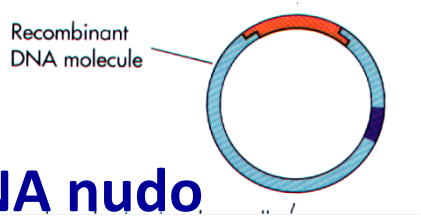
Vettori virali



Retroviruses
Lentiviruses
Adenoviruses
Virus adeno-associati (AAV)

Metodi fisici

Microiniezione
Elettroporazione
Gene Gun
DNA complessato a nanoparticelle



DNA nudo

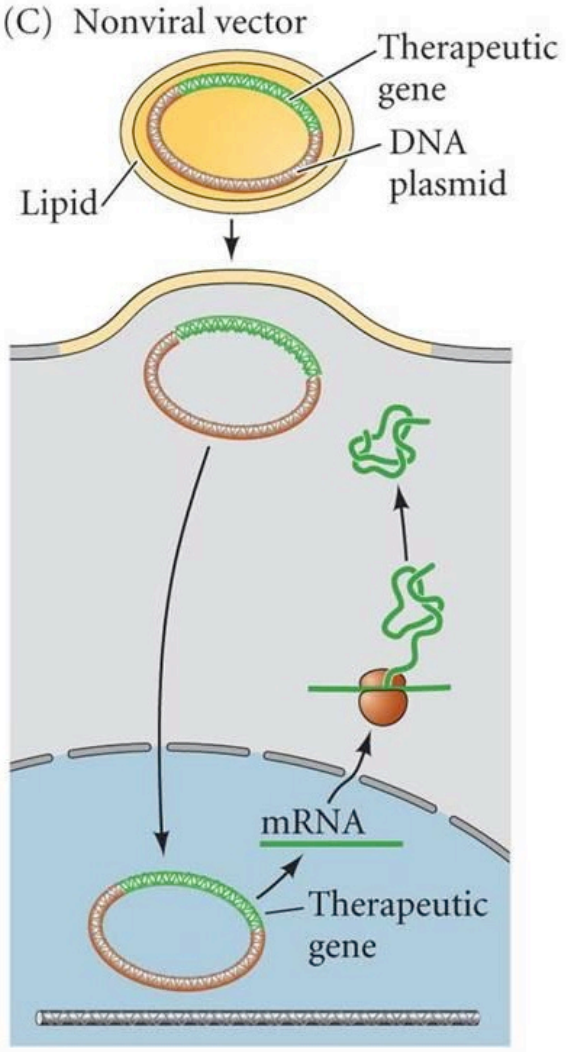
DNA nudo e metodi fisici

Vantaggi

- assenza di immunogenicità
- alta efficienza ex-vivo
- rilascio di grossi geni
- utile per le vaccinazioni a DNA

Svantaggi

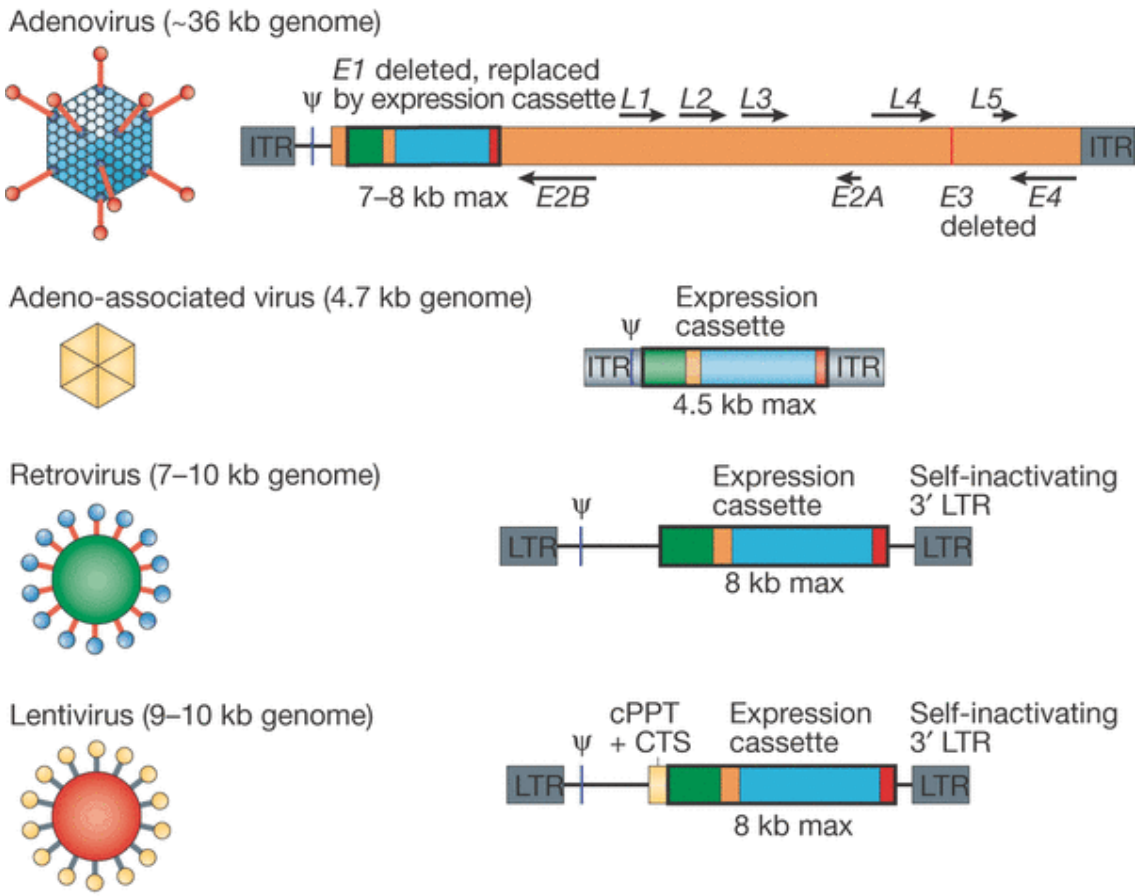
- espressione transitoria
- in vivo solo per tessuti superficiali (cute), muscolo, cuore, fegato



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



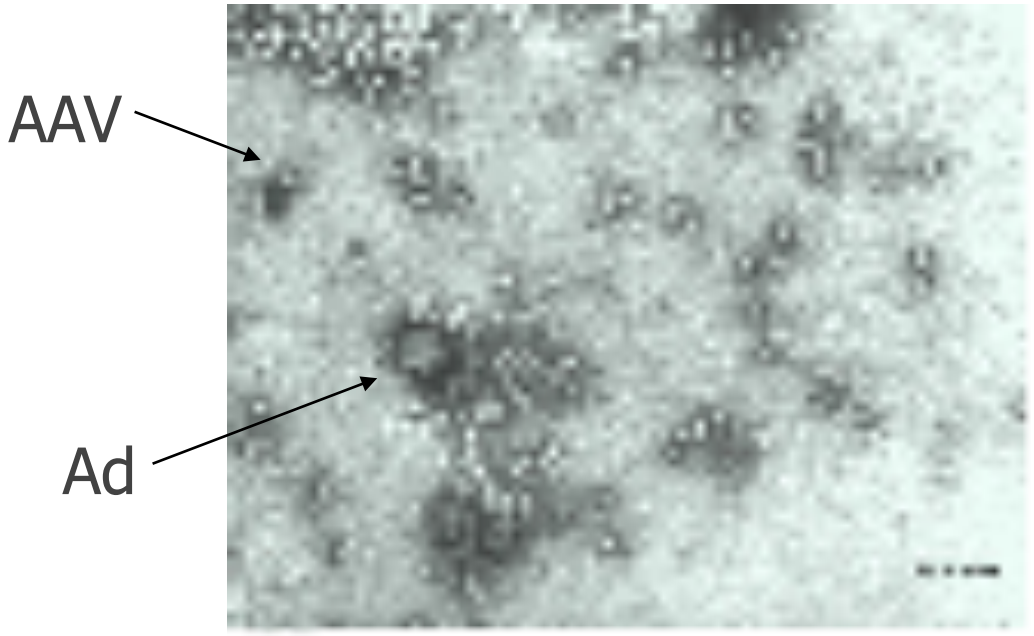
Vantaggi

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

Svantaggi

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

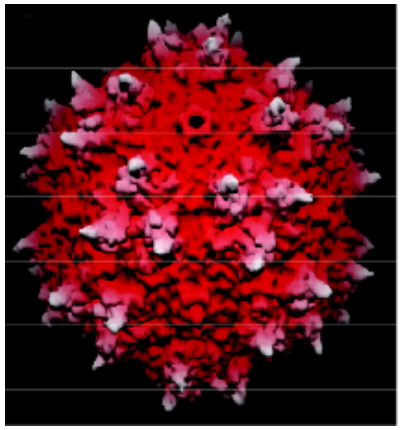
Adeno-associated virus (AAV)



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.

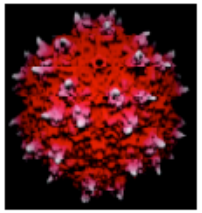
Genome
Linear, non-segmented, ssDNA ~5 kb.
AAVs package equal amounts of (+) and (-) strands.



Xie et al. 2002

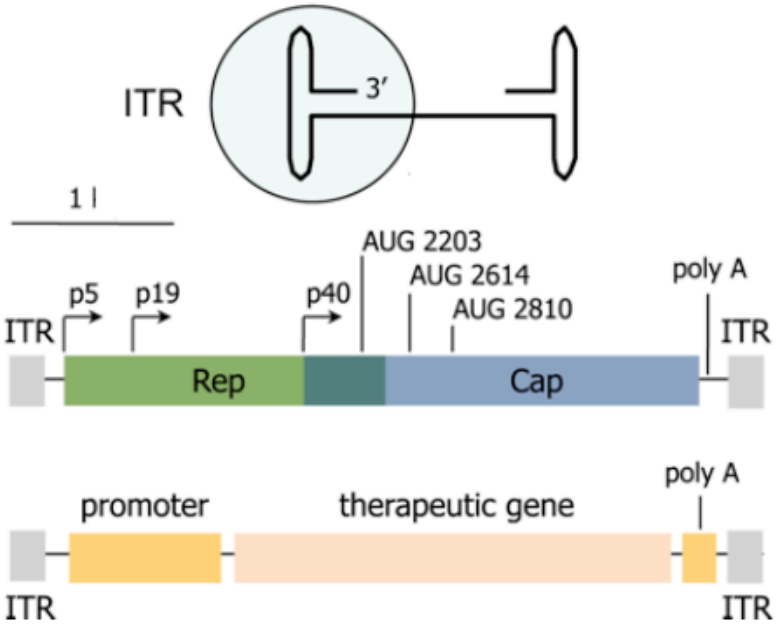
Gene transfer using Adeno-Associated Virus (AAV) vectors

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

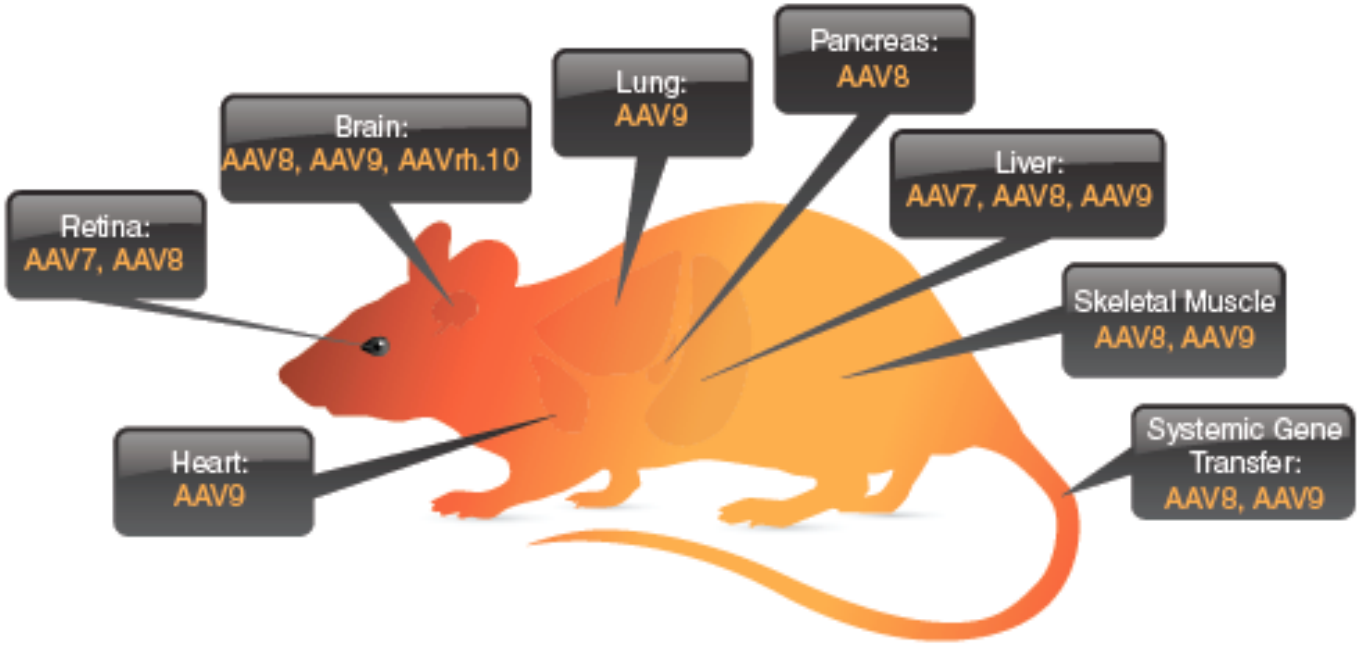


Xie et al. 2002

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

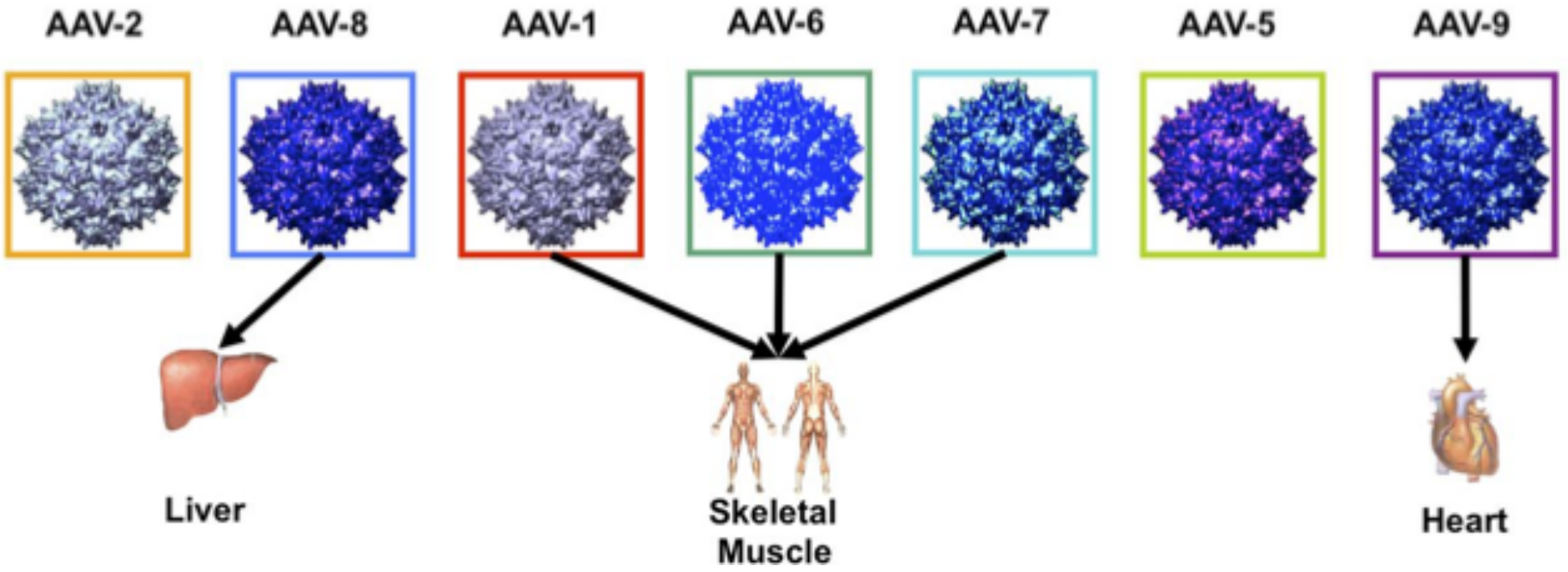


AAV efficiently transduces permissive tissues and promotes persistent transgene expression



Optimal AAV serotypes for in vivo gene transfer to various tissues

La tessuto-specificita’ di infezione e’ data dalle proteine del capside

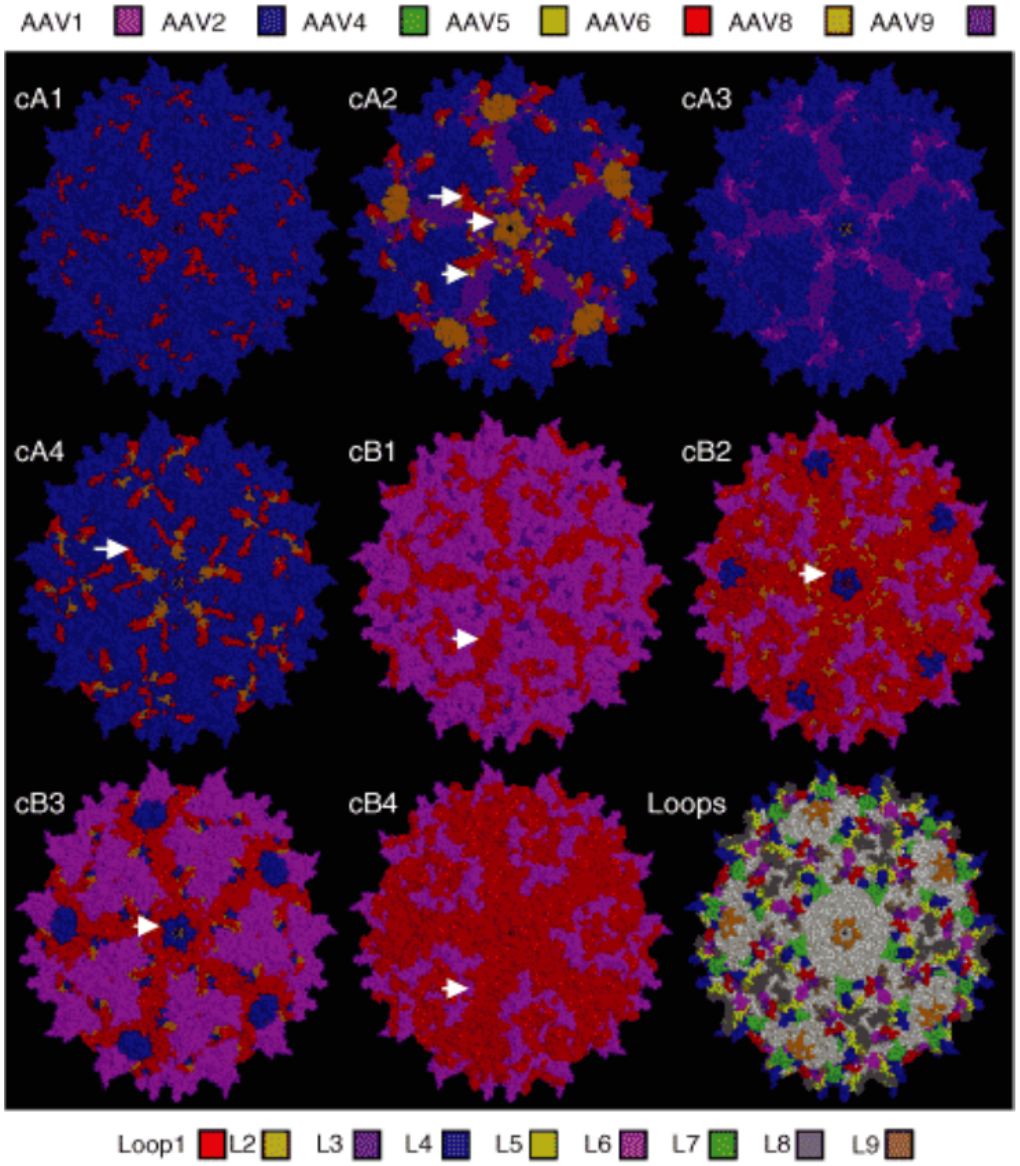


- RECETTORE?

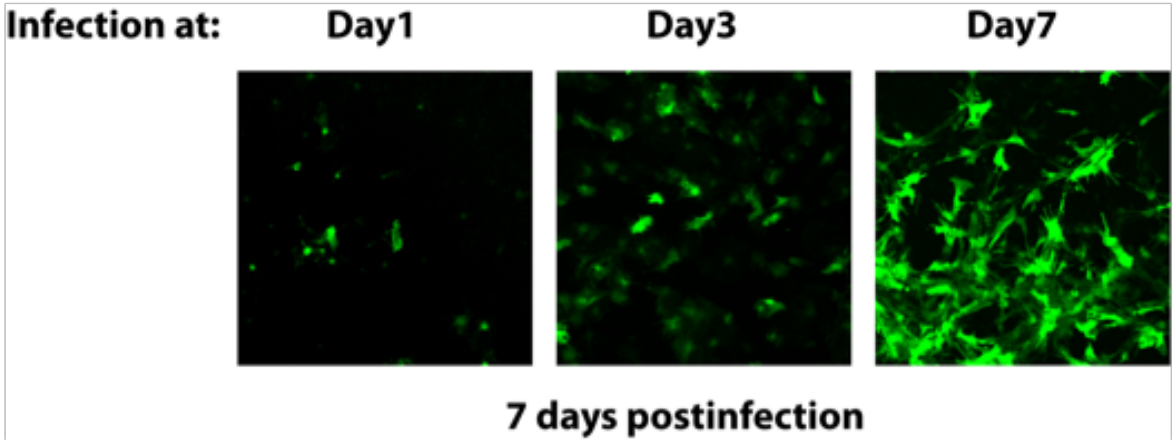
Recettori di alcuni Parvovirus

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

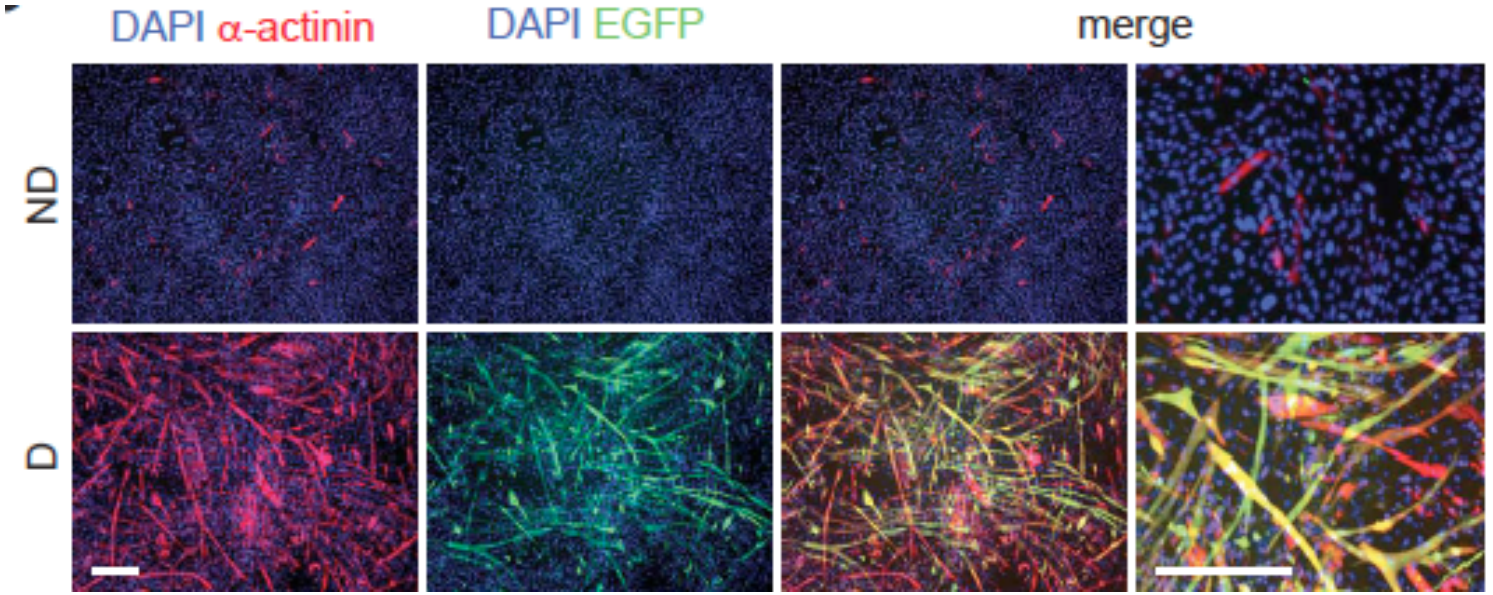
New capsid variant can be created by rational engineering or DNA shuffling



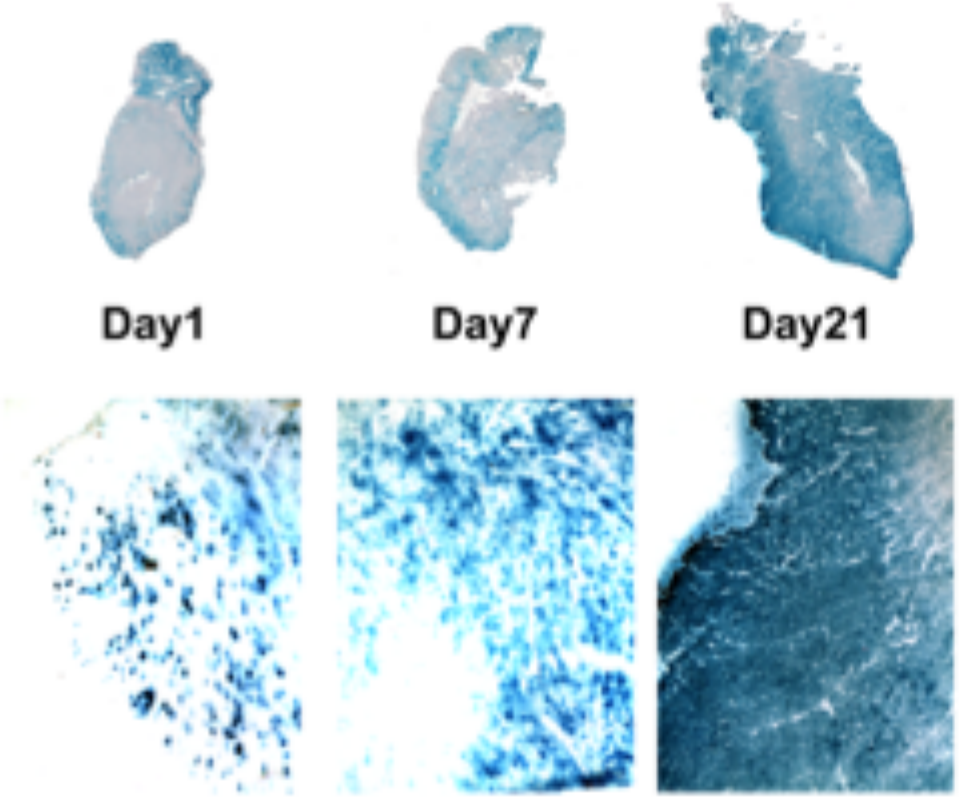
rAAV transduction increases with differentiation status of cultured cardiomyocytes

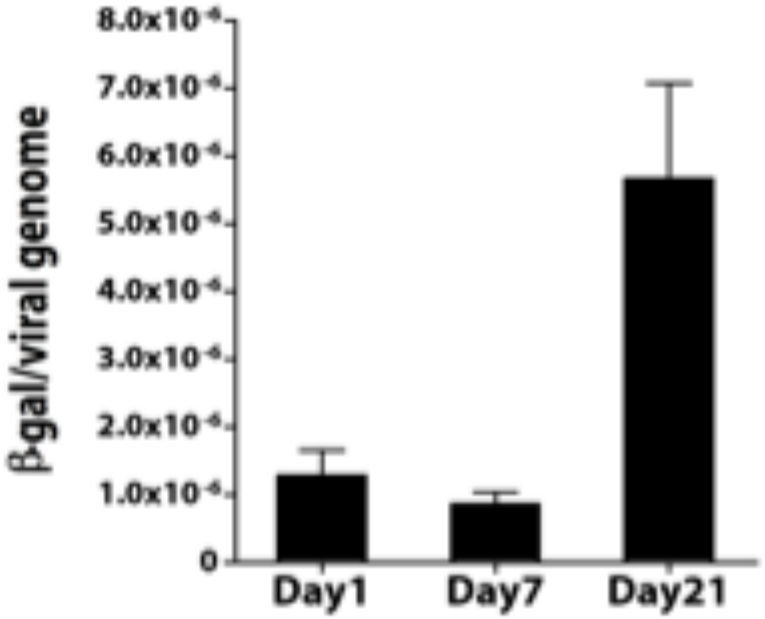


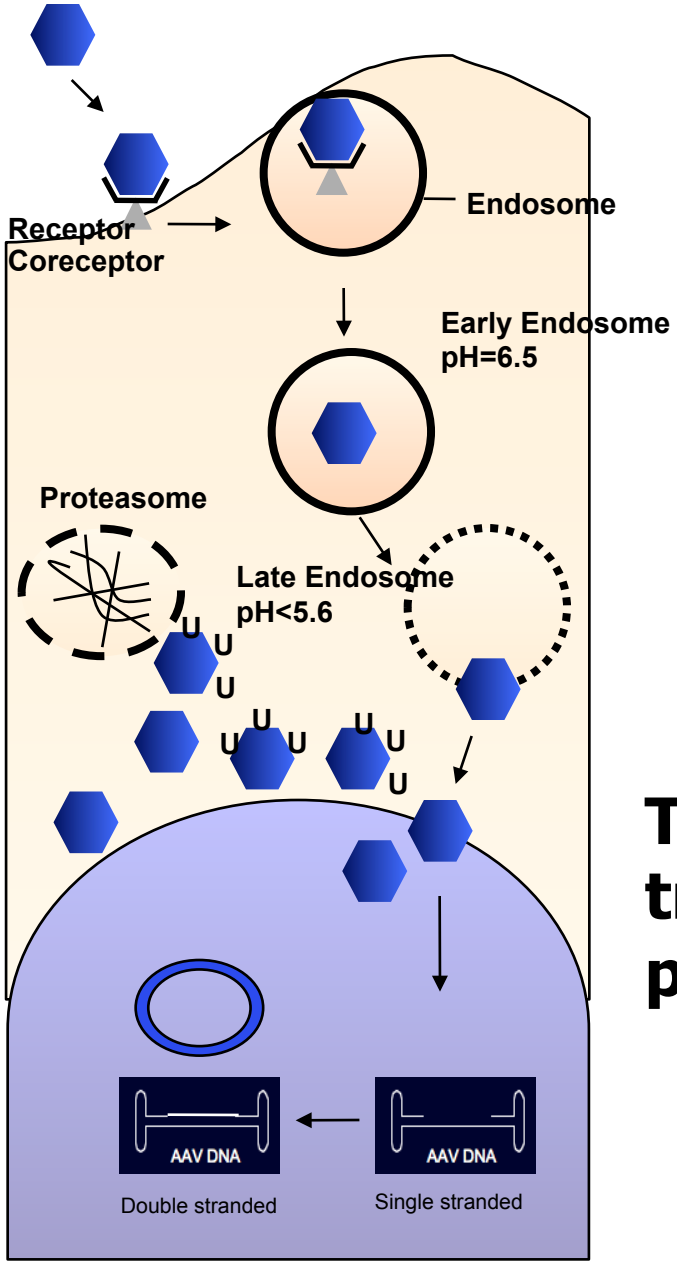
Permissivity of C2C12 cells to adeno-associated virus (AAV) transduction markedly increases during cell differentiation



Cardiomyocyte permissivity to in vivo AAV9 transduction increases after birth







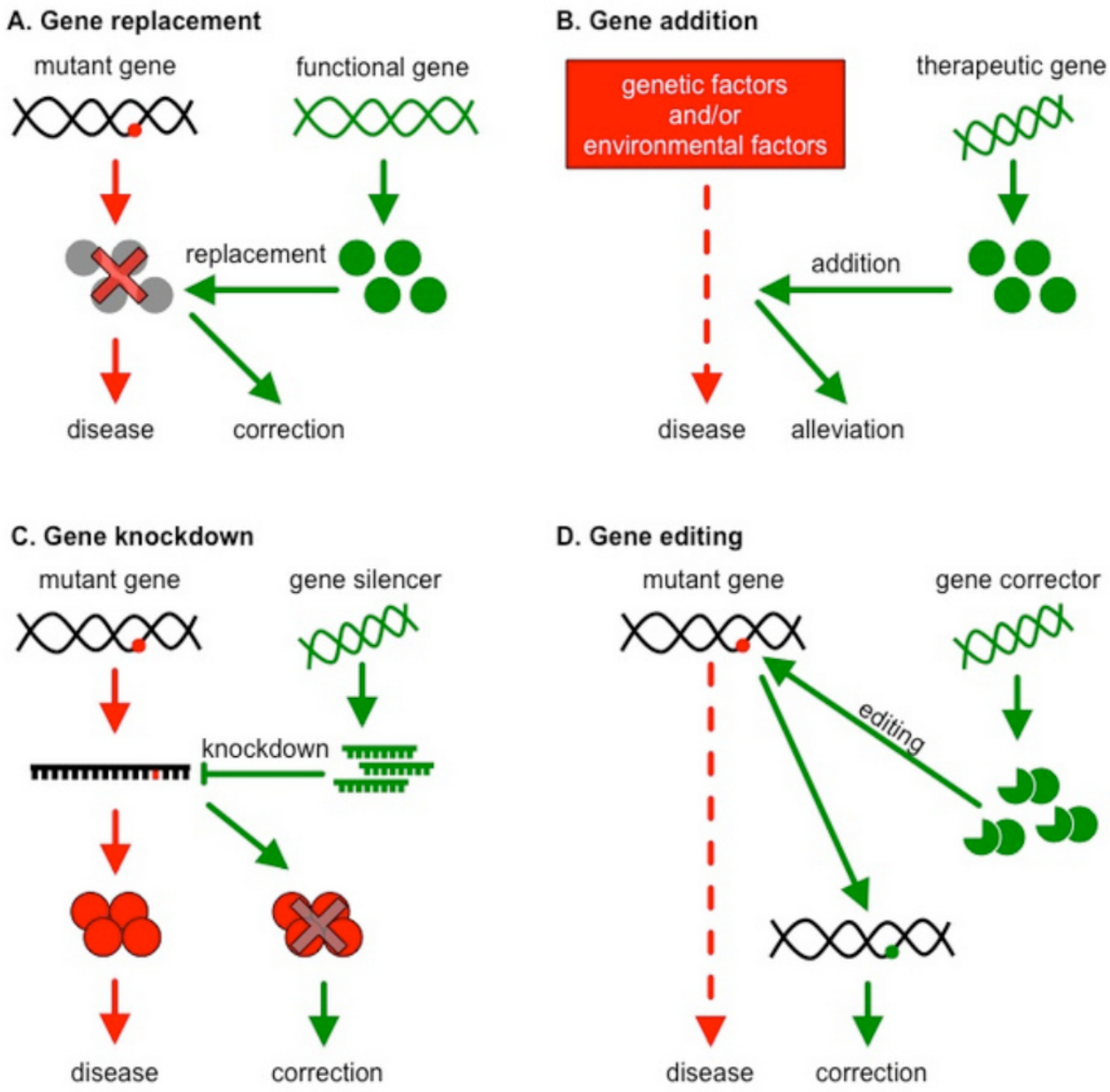
Infectious entry pathways of AAV vectors

- 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.

AAV-basedTherapy Strategies

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

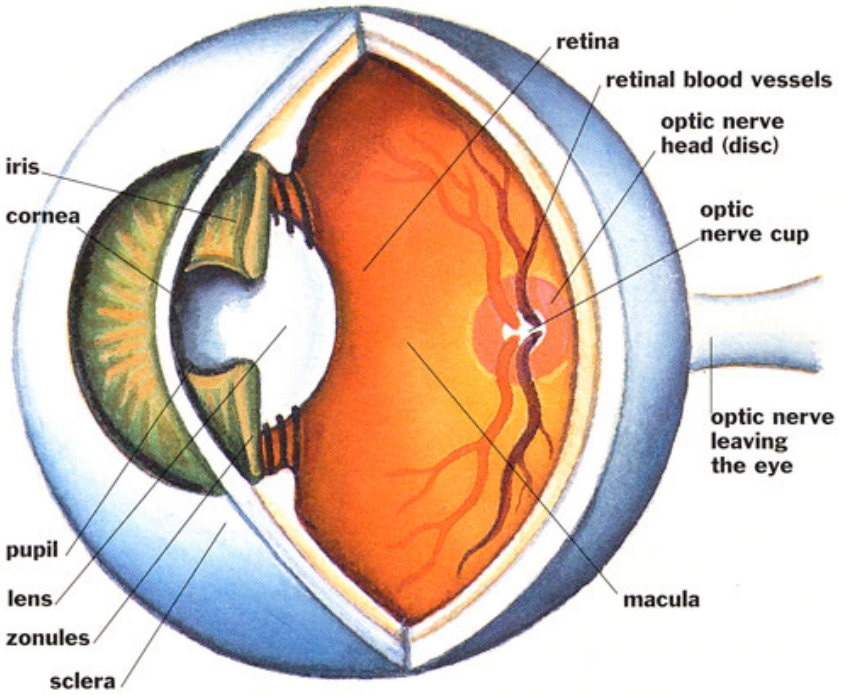
Most cells in the post-natal eye are terminally differentiated and prone to degenerative processes

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



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

AAV2 subretinal

AAV8 subretinal

AAV2 intravitreal

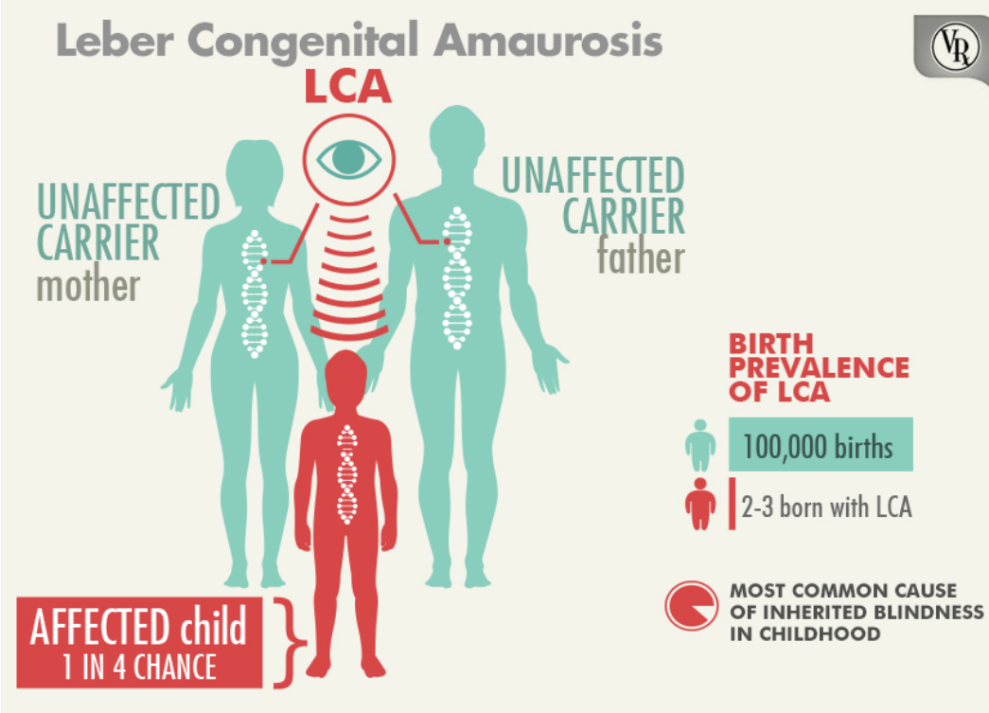
Amaurosi congenita di Leber

E' una delle forme più gravi di cecità congenita, presente alla nascita o nei primi mesi di vita.

Incidenza 3 casi ogni 100.000 nati vivi.

Malattia ereditaria a trasmissione autosomica recessiva. Sono stati individuati sei geni diversi, la cui mutazione porta al manifestarsi della malattia; si tratta di geni responsabili dello sviluppo, della funzionalità e del mantenimento della retina. Tuttavia questi geni rendono conto solo del 16% dei casi.

RPE65 codifica per retinolo cis/trans isomerasi dell' epitelio pigmentato



TERAPIA GENICA AAV-mediata

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

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- 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

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BRIEF REPORT

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

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- 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

Napoli, nuova terapia genica rende la vista a due bambini ciechi

La malattia è causata da mutazioni in un gene chiamato RP65 e la terapia fornisce una copia funzionante di questo gene

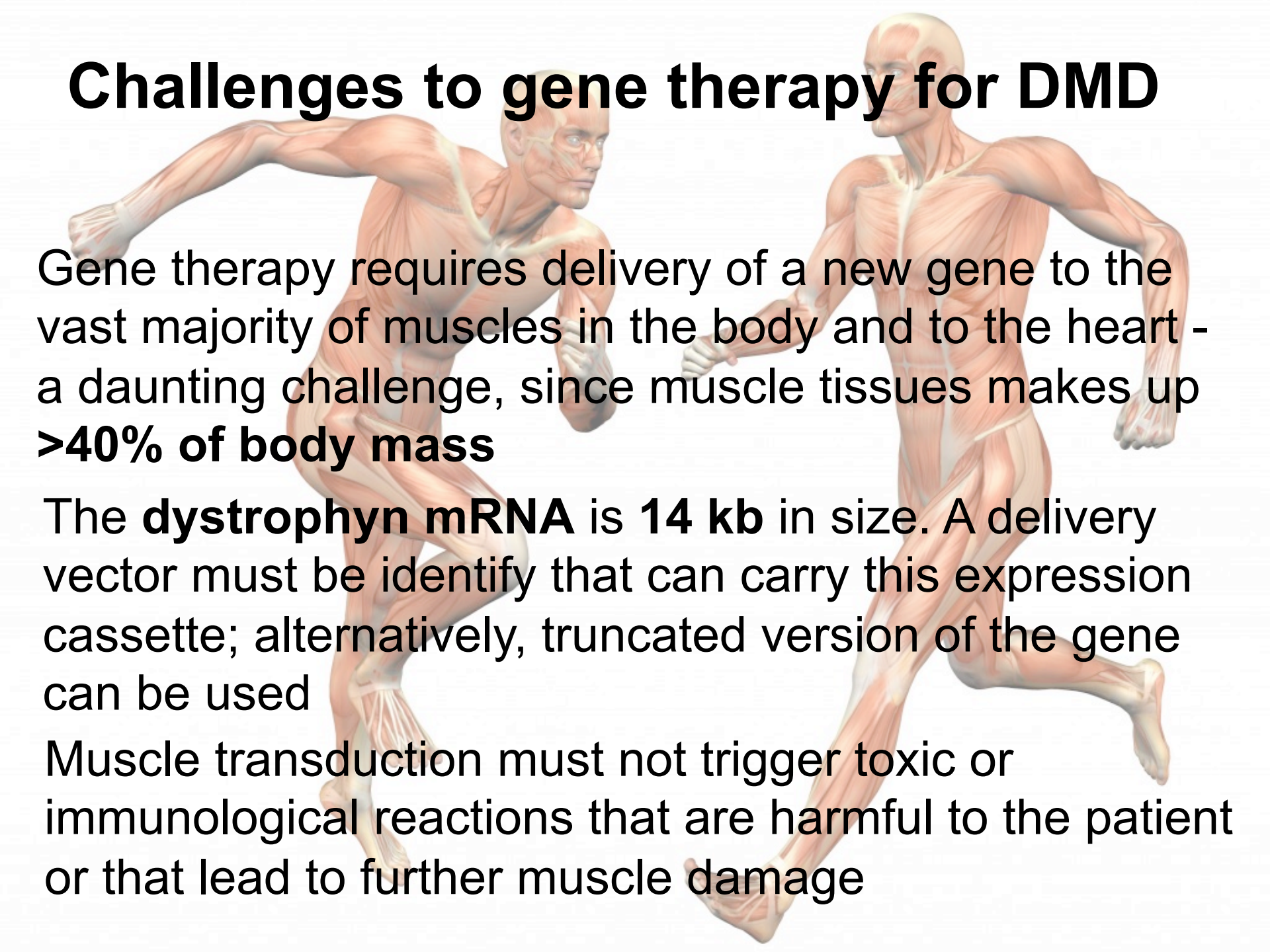
Due bambini, uno di 8 anni e uno di 9, **ciechi dalla nascita** per una particolare forma di **distrofia retinica ereditaria**, hanno recuperato la vista grazie a una tecnica innovativa eseguita **per la prima volta in Italia** nella Clinica oculistica dell'Università degli Studi della Campania "Luigi Vanvitelli". La malattia è causata da **mutazioni** in un gene chiamato **RP65** e la terapia, sviluppata da Novartis, fornisce una **copia funzionante** di questo gene.

I risultati ottenuti con la nuova metodologia, denominata **"Luxturna"**, sono stati illustrati da **Francesca Simonelli**, direttrice della Clinica Oculistica dell'ateneo. La copia del gene RP65 è in grado, attraverso una **singola somministrazione**, di migliorare la capacità visiva dei pazienti.

La tecnica e i risultati sono stati presentati dalla Simonelli presso il Policlinico di Napoli. Tra i partecipanti: Giuseppe Paolisso, Rettore dell'Università degli Studi della Campania Luigi Vanvitelli; Giuseppe Limongelli, direttore del Centro di coordinamento Malattie Rare della Regione Campania; Fulvio Luccini, Patient Access Head Novartis.

TGCOM24-16/12/2019

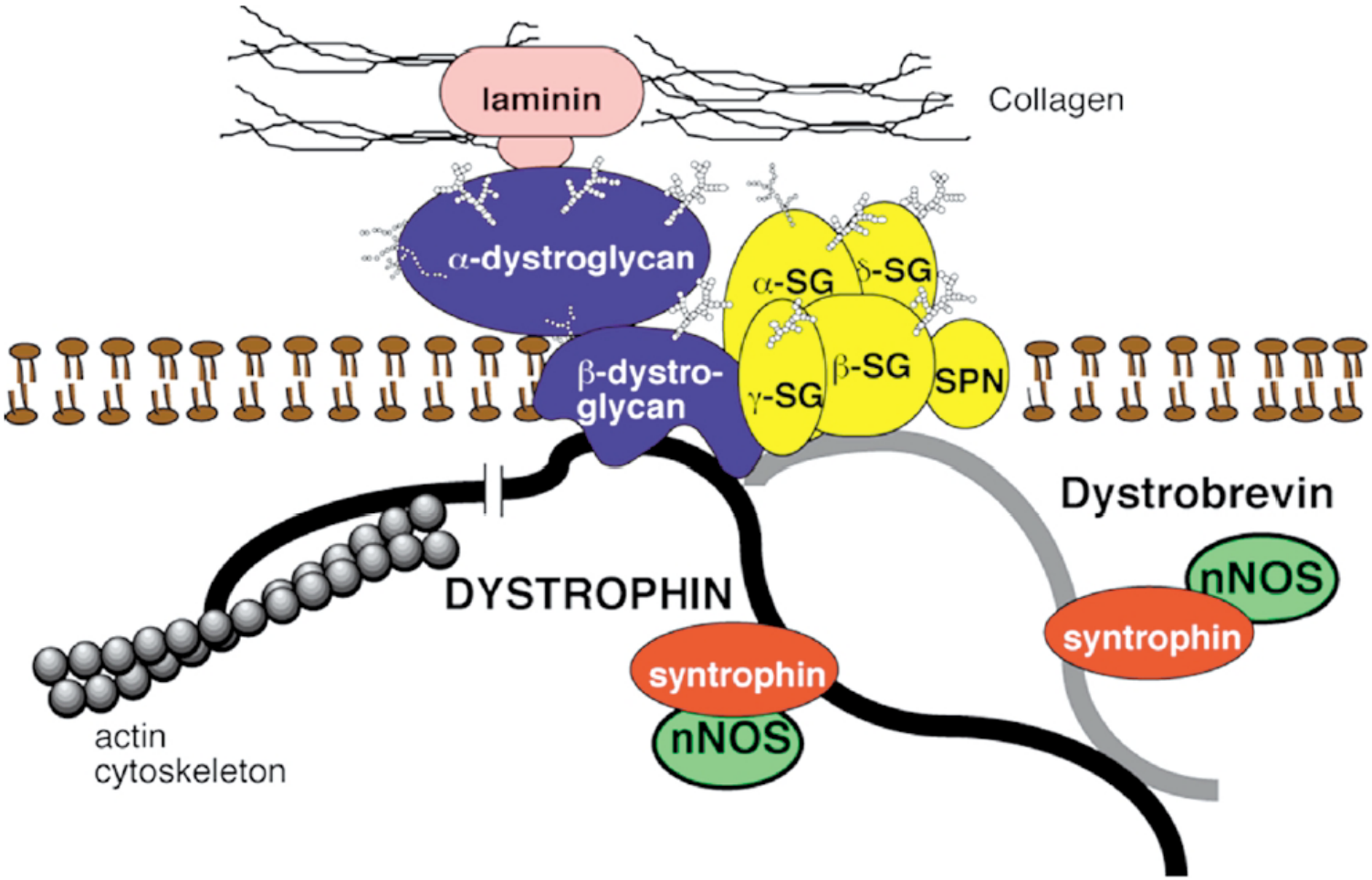
Challenges to gene therapy for DMD



Gene therapy requires delivery of a new gene to the vast majority of muscles in the body and to the heart - a daunting challenge, since muscle tissues makes up **>40% of body mass**

The **dystrophyn mRNA** is **14 kb** in size. A delivery vector must be identify that can carry this expression cassette; alternatively, truncated version of the gene can be used

Muscle transduction must not trigger toxic or immunological reactions that are harmful to the patient or that lead to further muscle damage


laminin
Collagen
α-dystroglycan
α-SG
δ-SG
β-dystro-
glycan
γ-SG
β-SG
SPN
Dystrobrevin
DYSTROPHIN
nNOS
syntrophin
syntrophin
nNOS
actin
cytoskeleton

Distrofina e DGC

R254 *Human Molecular Genetics, 2006, Vol. 15, Review Issue No. 2*

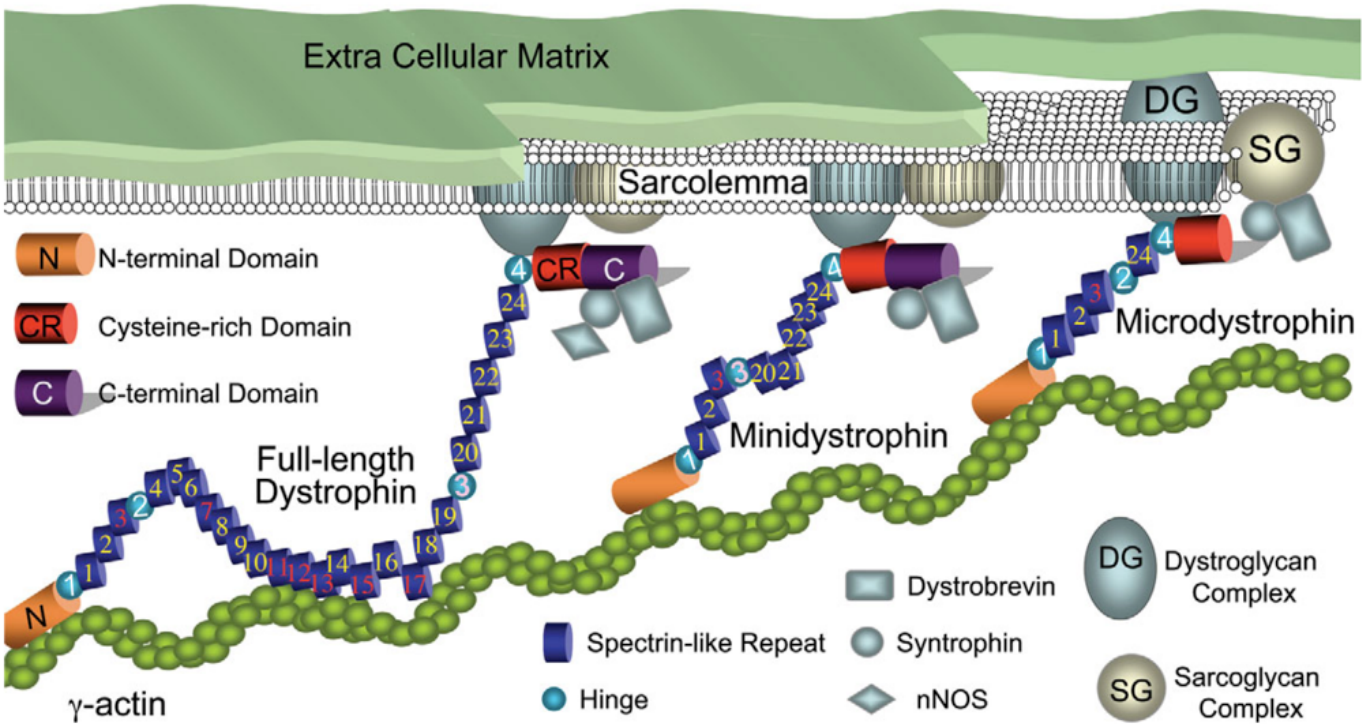


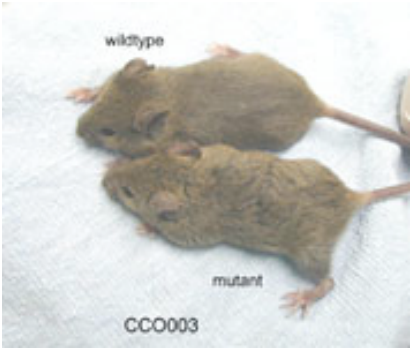
Figure 1. Schematic outline of full-length dystrophin, minidystrophin and microdystrophin and their interaction with other cellular proteins. Spectrin-like repeats are numbered from 1 to 24 (positively charged repeats are in red color, other repeats are in yellow color). Proline-rich hinges are numbered from 1 to 4. Hinge 3 is in pink color to indicate that it can be cleaved by viral protease. Hinge 2 to repeat 19 are deleted in minidystrophin. Repeat 4–23 and the C-terminal domain are deleted in microdystrophin. Not drawn to scale.

minidistrofine (~6-7 kb) e microdistrofine (~4 kb)

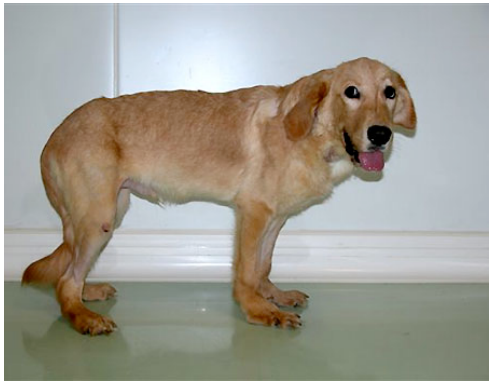
Queste versioni ridotte della distrofina presentano delezioni comuni della regione centrale a bastoncello e nel dominio C-terminale della proteina parentale, lasciando intatti i domini funzionali essenziali della proteina, in particolare quello ricco in cisteine (CR)

Duchenne muscular dystrophy - natural animal models

mdx mice (point mutation leading to a premature truncation)



xdm golden retriever dog (exon 7 skipping)

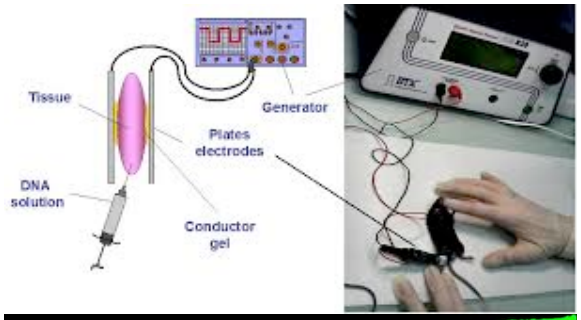


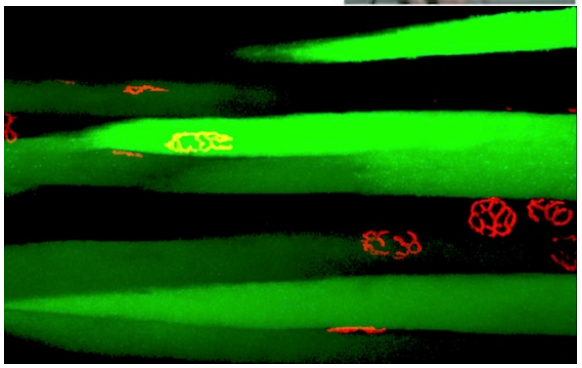
Vectors for muscle gene therapy

• ***Ad "gutless":*** Ad vectors have relative large cloning capacity, can be grown at high titers and display relative efficient infection of muscle. However, traditional Ad vectors elicit a robust cellular immune response

• ***AAV:*** AAV vectors are of great interest as they efficiently infect muscle and persist for years. New serotypes available

• ***Plasmid DNA:*** displays a remarkable ability to transfer genes to muscle, specially if coupled with high pressure injection and/or electroporation





The first clinical trial, closed in 2006, entailed the injection of a plasmid containing the whole dystrophin cDNA under the control of the CMV promoter into the radialis muscle of 9 DMD/BMD patients. However, dystrophin expression resulted too low and not homogenous.

Different strategies can be used to increased transduction efficiency, including polymeres, ultrasounds (with microbubbles), and electroporation

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

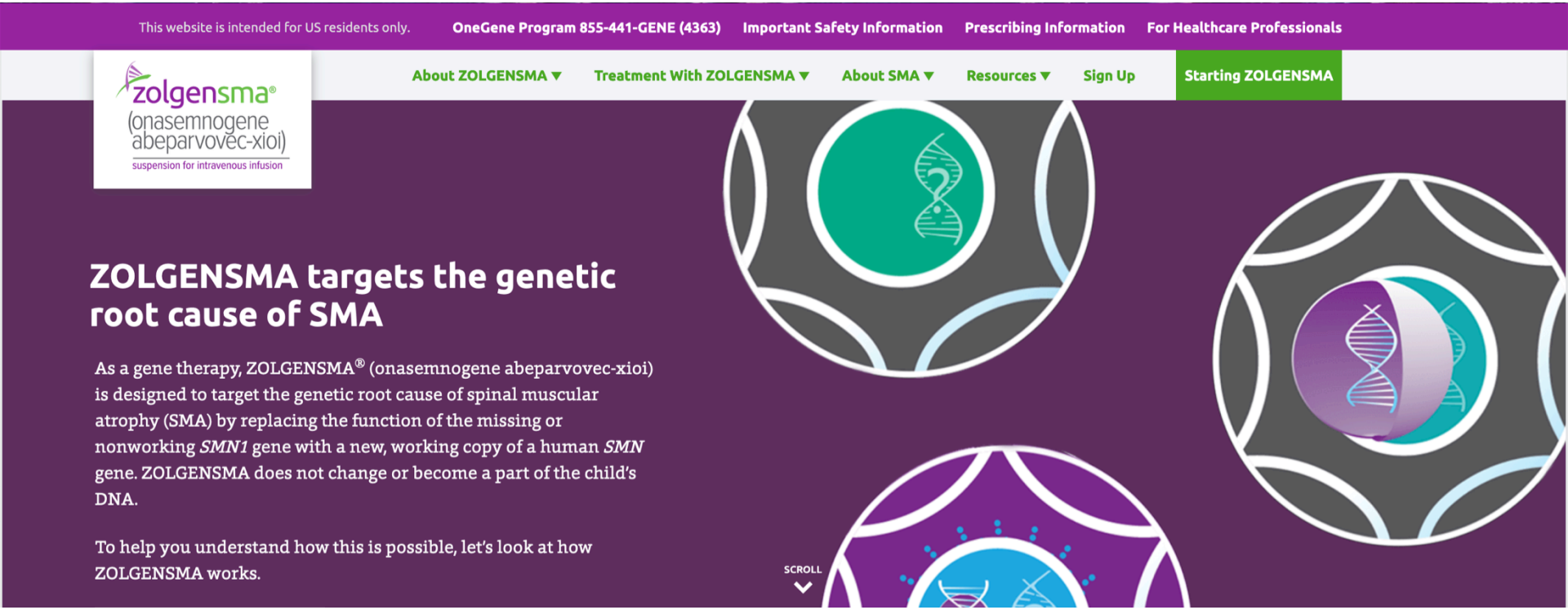
Rottamazione, «saldo e stralcio»: nuove scadenze per 1,2 milioni di italiani

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

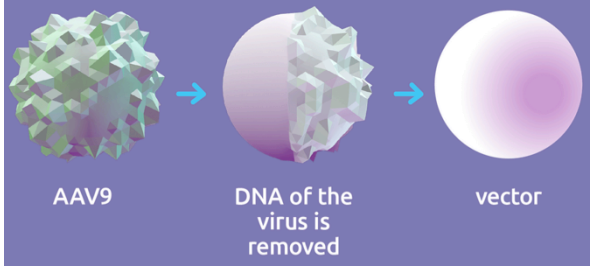
ZOLGENSMA targets the genetic root cause of SMA by replacing the function of the missing or nonworking gene, called the *SMN1* gene. This gene is critical to making SMN protein.

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.

Patients treated after SMA symptoms appeared (symptomatic)

STR1VE study (completed)

This study followed the START study.

Participants: 22* patients with SMA Type 1 (had 2 copies of *SMN2* backup gene, showed symptoms of SMA, and were 6 months of age or younger at the time of infusion)

Goal: Establish efficacy and safety of ZOLGENSMA

*One patient was initially not part of the data set but is included in the final data analysis.

START study (completed)

This was the first study of ZOLGENSMA.

Participants: 15 patients with SMA Type 1 (had 2 copies of *SMN2* backup gene, showed symptoms of SMA, and were 8 months of age or younger at the time of infusion)

Goal: Establish safety and determine the appropriate dose of ZOLGENSMA

START LTFU study (ongoing)

In addition, participants in the START study were invited to voluntarily enroll in a long-term follow-up (LTFU). The ongoing 15-year observational study enrolled 13 of 15 patients from the START study and is intended to monitor ongoing safety.

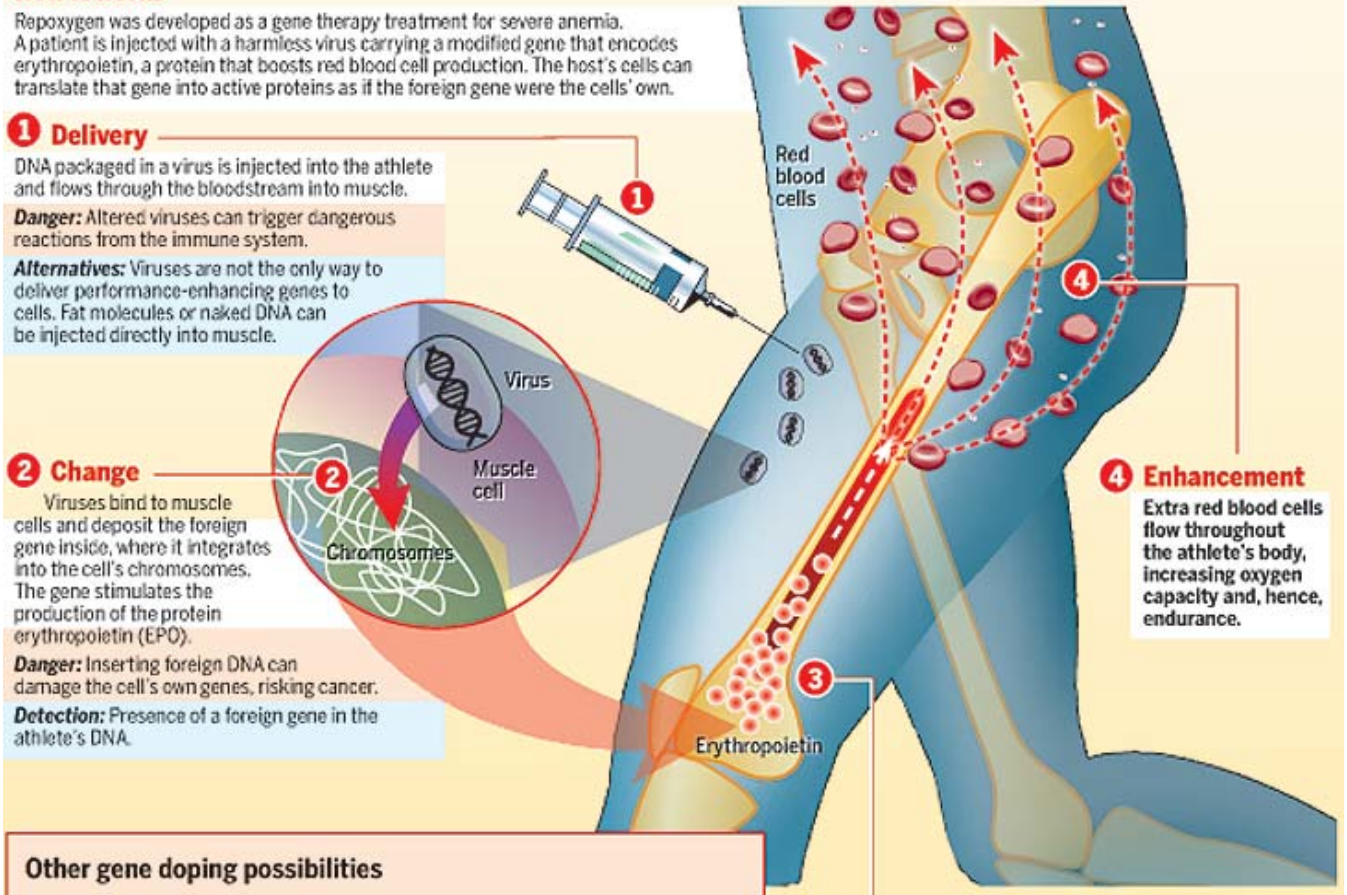
https://www.zolgensma.com/clinical-studies/symptomatic-study-results

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.

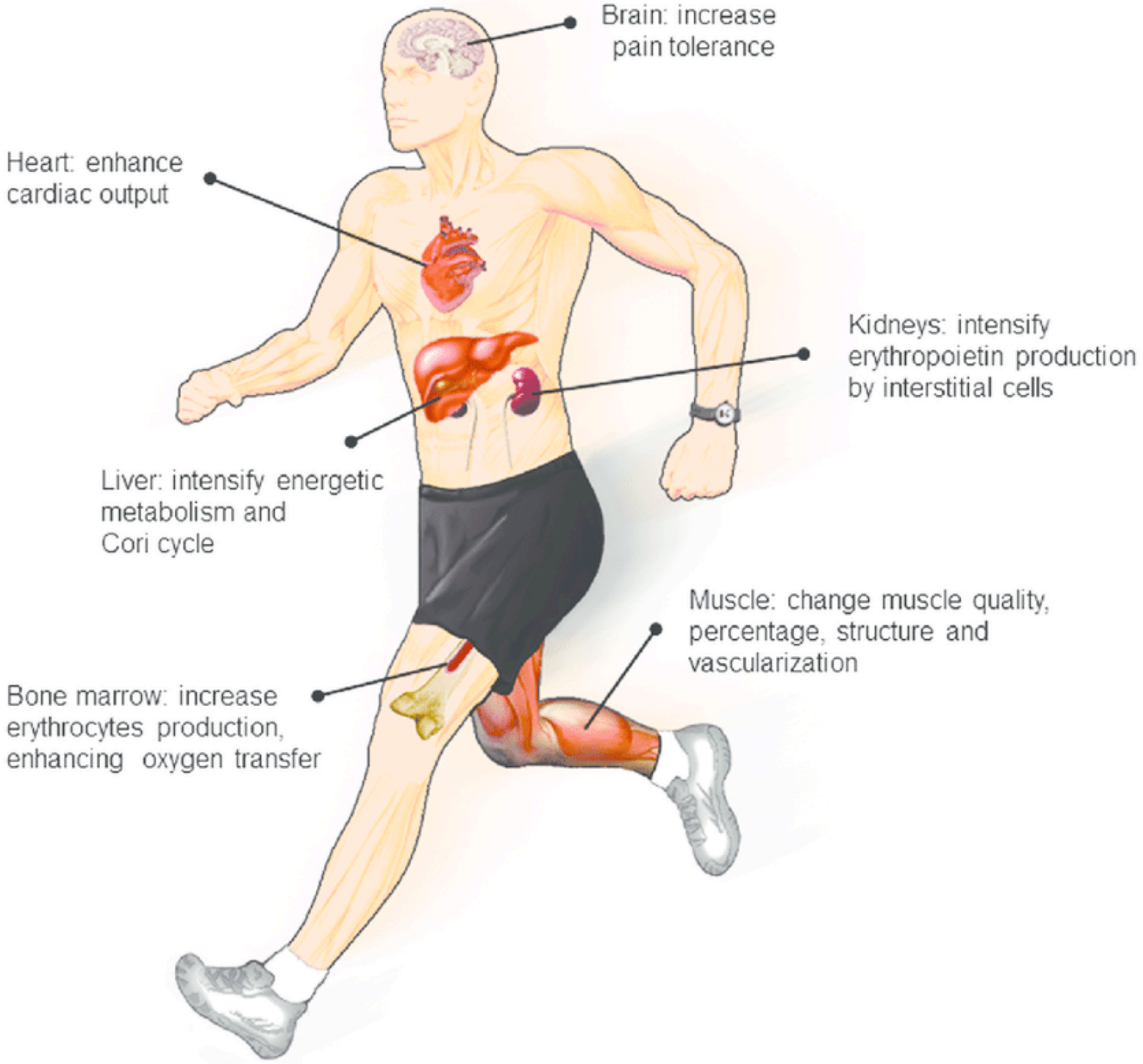
SOURCE: *Finding the Golden Genes*, Patrick Barry, www.sciencenews.org

SUSIE MAH/ SUN MEDIA

WORLD ANTI-DOPING AGENCY

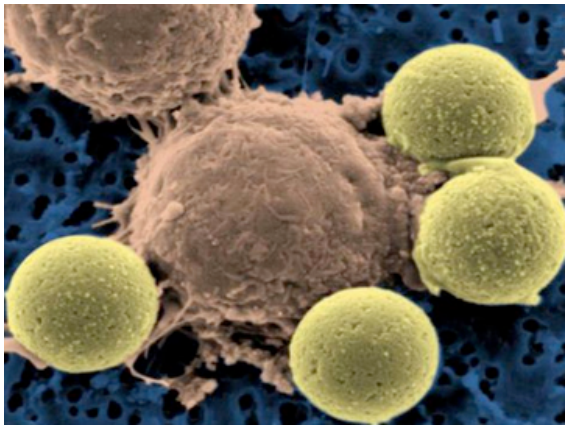
Geni:

-EPO
-IGF1
-Inibitori della Miostatina


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

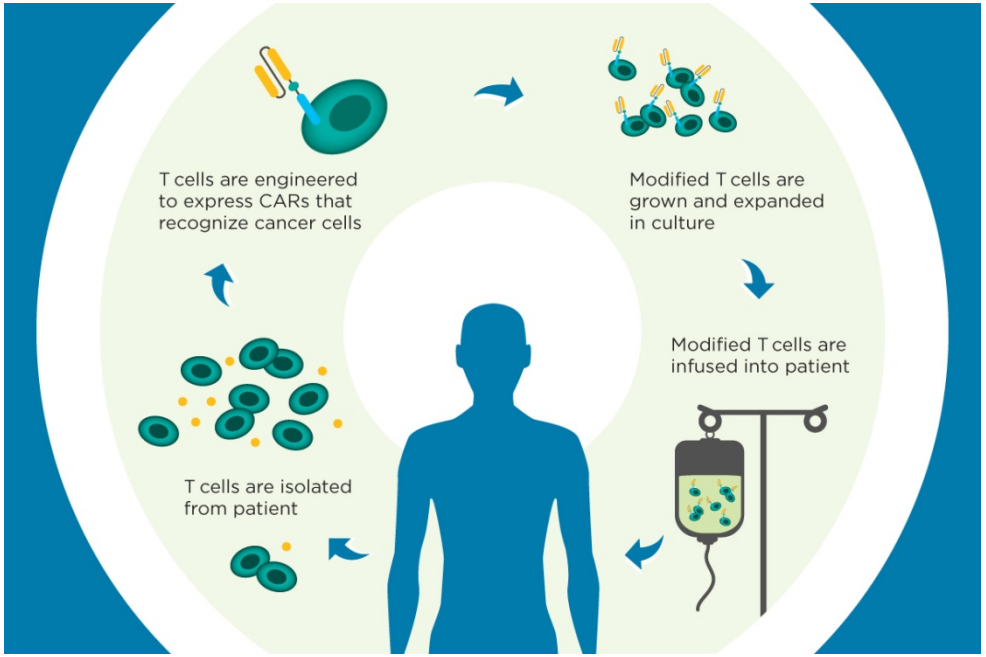
CAR-T cell therapy: Engineering Patients' Immune Cells to Treat Their Cancers

Chimeric antigen receptors (CARs)
Adoptive cell transfer is like "giving patients a living drug,"

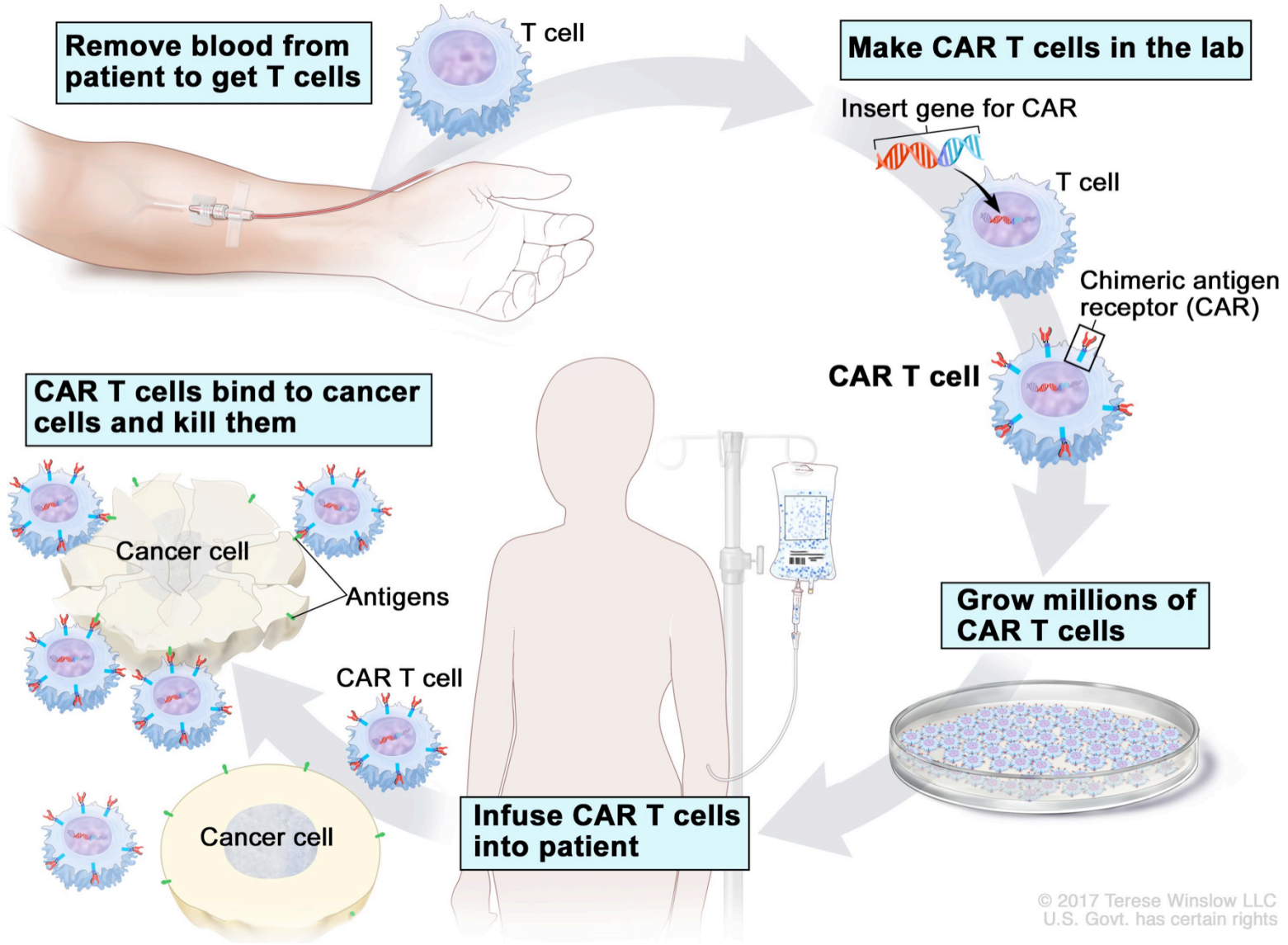


Immunotherapy—therapies that harness the power of a patient's immune system to combat their disease,

One approach : immunotherapy involves engineering patients' own immune cells to recognize and attack their tumors-remarkable responses in patients with advanced cancer.



Overview of CAR T-cell therapy in the clinic.



A patient's T cells are harvested through leukapheresis, followed by T-cell activation on antibody-coated beads. The activated T cells are then genetically reprogrammed *ex vivo* by transduction with a construct encoding the CAR, and the CAR T cells are further expanded *ex vivo*. When the CAR T-cell product has been prepared and has passed all quality control testing, the patient receives lymphodepleting chemotherapy and CAR T-cell infusion. © Novartis Pharmaceuticals.

Le CAR-T autorizzate.

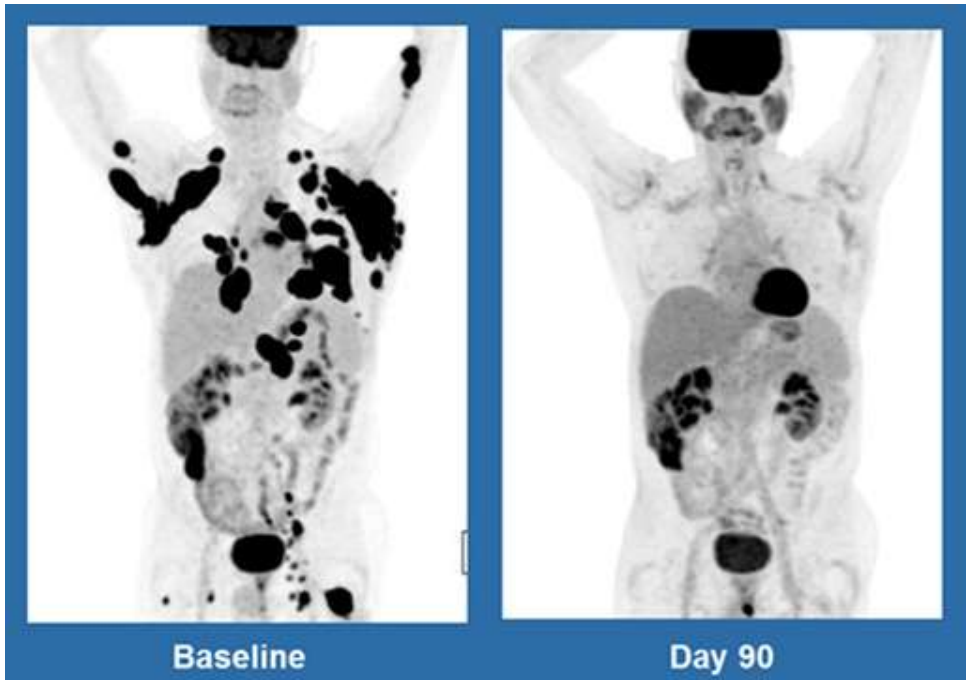
Le terapie CAR-T rappresentano la prima forma di terapia genica approvata per il trattamento della **leucemia linfoblastica B** e di alcune forme aggressive di **linfoma non-Hodgkin.**

- Kymriah (tisagenlecleucel), autorizzato il 22 agosto 2018
- Yescarta (axicabtagene ciloleucel), autorizzato il 23 agosto 2018,

indicate per il trattamento di:¬ pazienti pediatrici e giovani adulti fino a 25 anni di età affetti da leucemia linfoblastica acuta a cellule B che non abbiano mai risposto alla chemioterapia, o che siano in recidiva dopo trapianto di cellule staminali emopoietiche allogeniche o dopo almeno 2 linee di chemioterapia (Kymriah); pazienti con linfoma diffuso a grandi cellule B o DLBCL (Kymriah e Yescarta) /linfoma primitivo del mediastino a cellule B o PMBCL (Yescarta) già sottoposti ad almeno 2 linee di terapia sistemica.

CAR-T: efficacia e sicurezza del trattamento

Rispetto alle terapie “convenzionali”, le CAR-T permettono di ottenere remissioni complete anche in fasi di malattia molto avanzate. Inoltre, a un anno dall’infusione di CAR-T, la maggior parte dei pazienti che ha ottenuto una remissione è ancora viva e libera da malattia.



These scans show a 62-year-old man with **non-Hodgkin lymphoma, at left in December 2015, and three months after treatment with Kite Pharma's experimental gene therapy at MD Anderson Cancer Center in Houston**. The treatment, called CAR-T cell therapy, turns a patient's own blood cells into specialized cancer killers. It worked in a study, with more than one third of very sick lymphoma patients showing no sign of disease six months after a single treatment, its maker said Tuesday, Feb. 28, 2017. The scans are from a presentation by Drs. Fred Locke and Sattva Neelapu, provided by the American Society for Blood and Marrow Transplantation and Kite. (ASBMT/ Kite Pharma via AP)

https://medicalxpress.com/news/2017-02-gene-therapy-blood-cancer-major.html#jCp

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