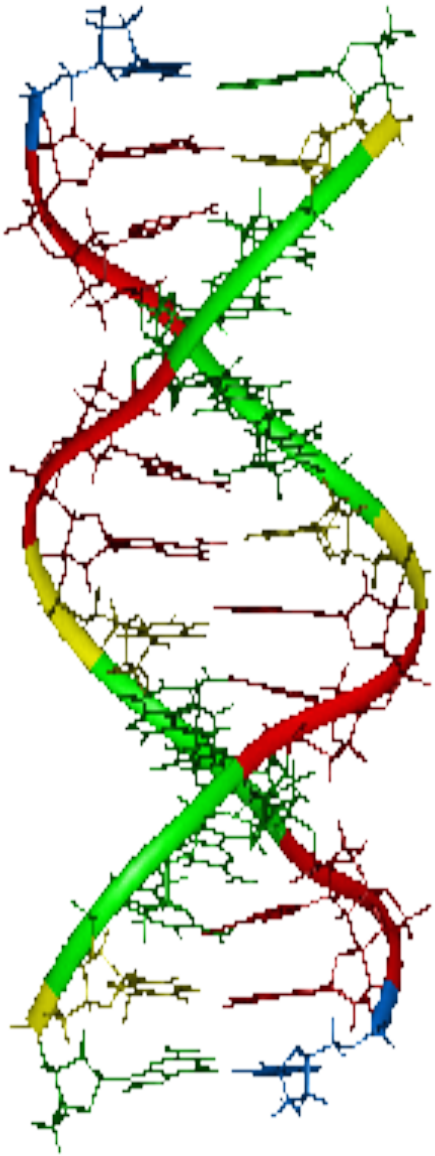


Terapia in genetica medica

Modelli di terapia genica

- ✓ Definizione e background
- ✓ Sistemi di rilascio
- ✓ Strategie di terapia genica
- ✓ Esempi di trials clinici nella distrofia muscolare
- ✓ Prospettive future

Terapia Genica: perché ?

Molte patologie, dovute a proteine malfunzionanti,
non sono trattabili con terapie tradizionali

Negli anni ‘70 nasce l’idea della **Terapia Genica**

(rilascio intracellulare di materiale genetico
per generare un effetto terapeutico, con diverse strategie di
intervento a seconda dello scopo prefissato)

Patologie bersaglio

- **Monogeniche**

  Immunodeficienze - Distrofia muscolare - Fibrosi cistica - Emofilie Retinopatie - Emoglobinopatie - Ipercolesterolemia fam - Xeroderma pigmentosum

- **Multifattoriali**

  Malattie cardiovascolari e neurodegenerative - Diabete - Artrite reumatoide

- **Tumorali**

  Leucemie - Carcinomi

- **Infettive**

  AIDS - Epatite B e C

- **Acquisite**

  Traumi (fratture ossee, ferite, ustioni) - Ischemie

Terapia genica: quale tipo?

SOMATICA

manipolazione dell'espressione genica in cellule differenziate dell'individuo adulto

l'alterazione genica riguarda esclusivamente il paziente su cui è stata realizzata

GERMINALE

manipolazione dell'espressione genica in cellule riproduttive

eventuali modificazioni geniche verrebbero trasmesse alla progenie

non autorizzata !!!!

Terapia genica: come?

ex vivo

Le cellule bersaglio (es. SC) 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 (es. FC); tessuto cutaneo ecc…

in vivo

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

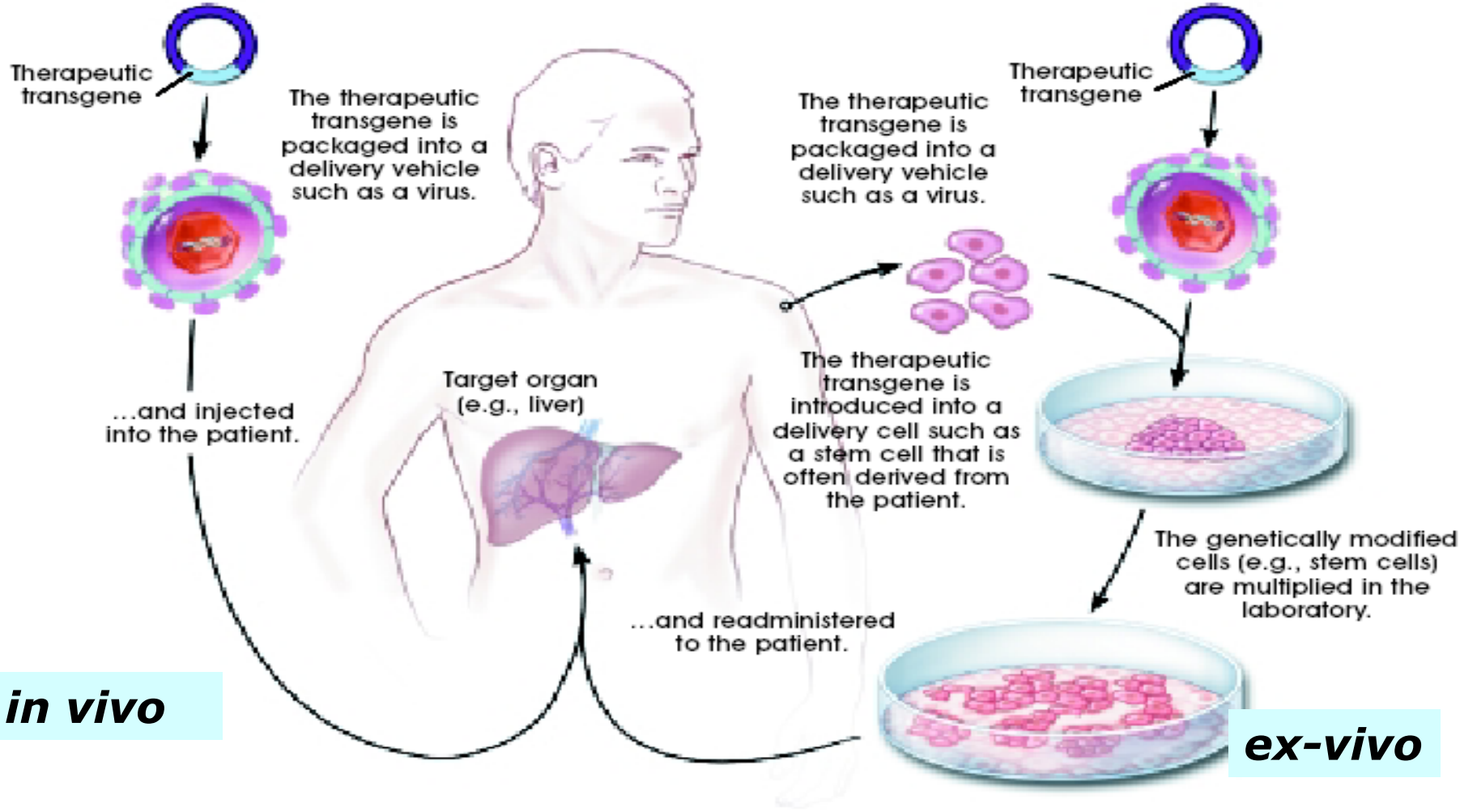
✓ cellule e tessuti poco accessibili

✓ scarsa efficienza di trasduzione, barriere

TERAPIA GENICA: come ?

Rilascio gene modificato

Rilascio cellule modificate

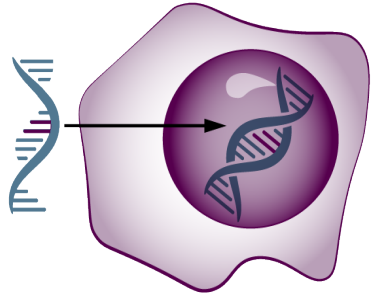


in vivo

ex-vivo

What Is Gene Therapy?

- Gene therapy is a technique that adds new genes or modifies a person's genes to treat or cure disease[1]
- Types of gene therapy:

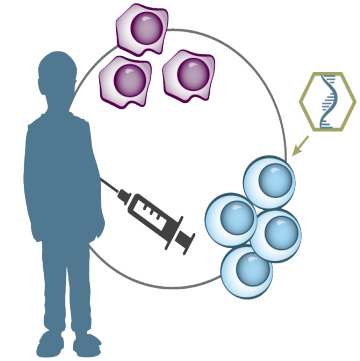


GENE TRANSFER
add a new gene

- Introduces a new or modified gene into the body to help treat the disease[1]

GENE EDITING
repair the defective gene

- CRISPR/Cas9[1]

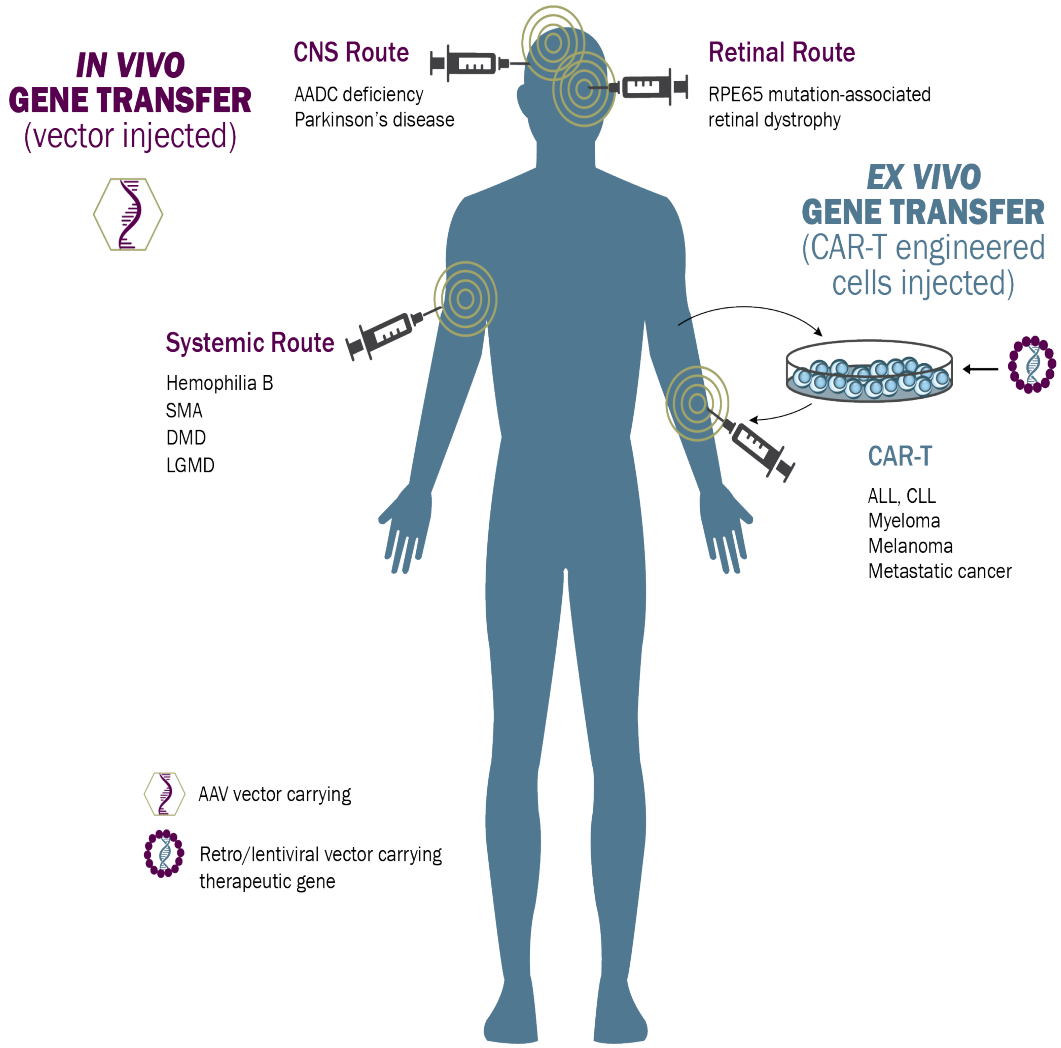


EX VIVO GENE THERAPY
modify cells outside the body

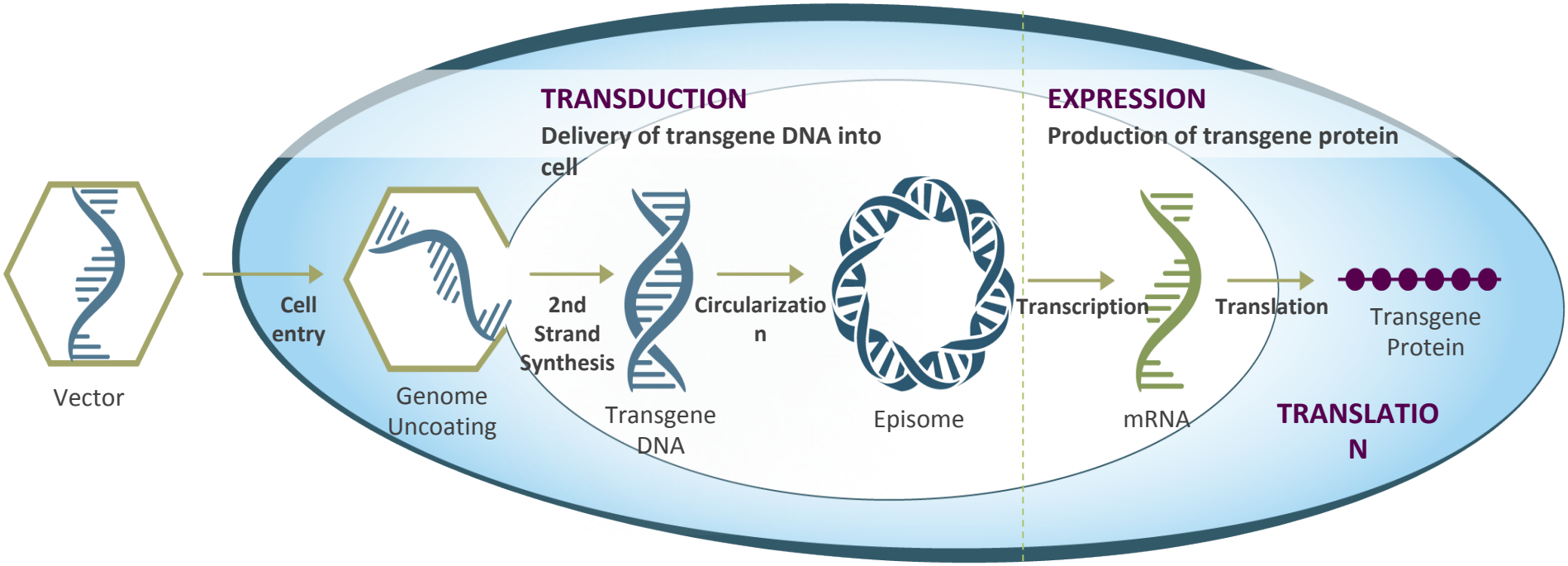
- CAR-T anticancer therapies[1]

Gene Transfer

- Vector
  - Modified virus that contains new gene
- Injected systemically or locally
  - Retinal, intramuscular, intrathecal, systemic
- Vector transfers gene into target tissue



Gene Therapy: Transduction and Expression



The tropism of the **vector** controls which cells are **transduced** with the transgene **DNA**

The transgene **promoter** controls which cells express the transgene **protein**

Trasferimento Genico *in vivo*

Aerosol

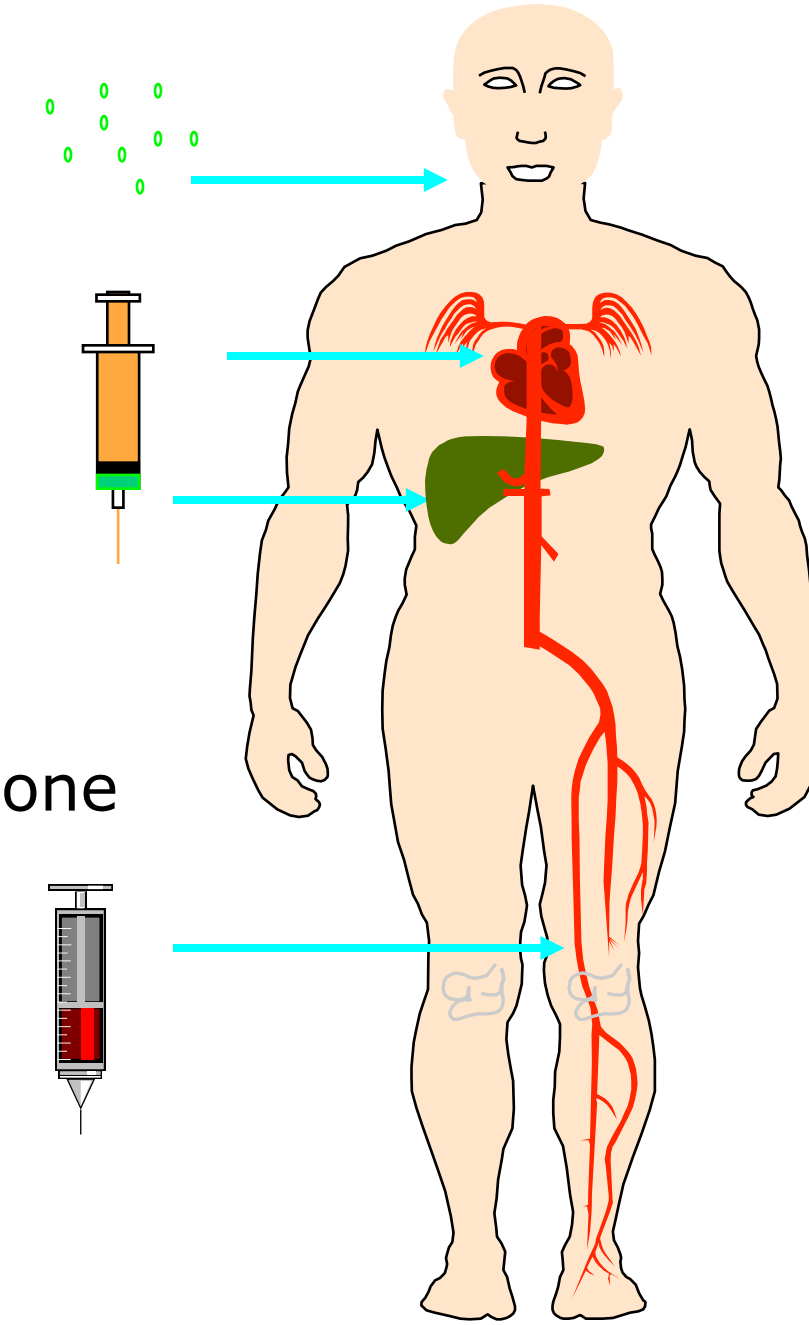
Iniezione diretta
(miocardio)

Perfusione organo

Uso di cateteri
(tumori solidi)

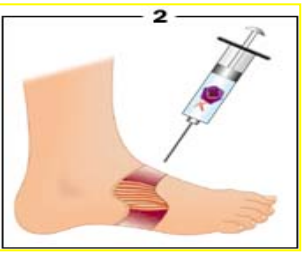
Gene gun/elettroporazione
(miofibrille, epidermide)

Via sistemica

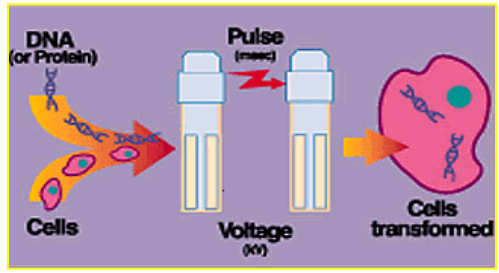


TERAPIA GENICA: come ?

DNA nudo



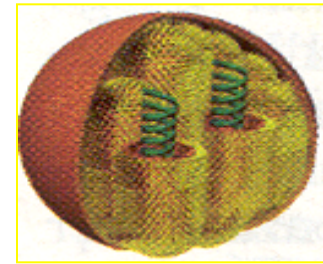
elettroporazione



pistola genica

microiniezione

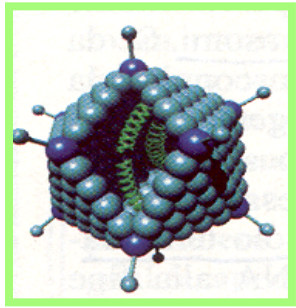
liposomi



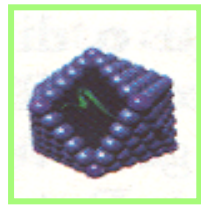
non-virali

Vettore per rilasciare e proteggere il gene terapeutico

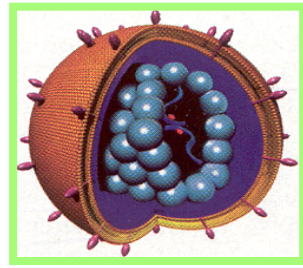
virali



adenovirus

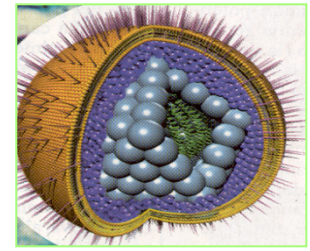


adenoassociati



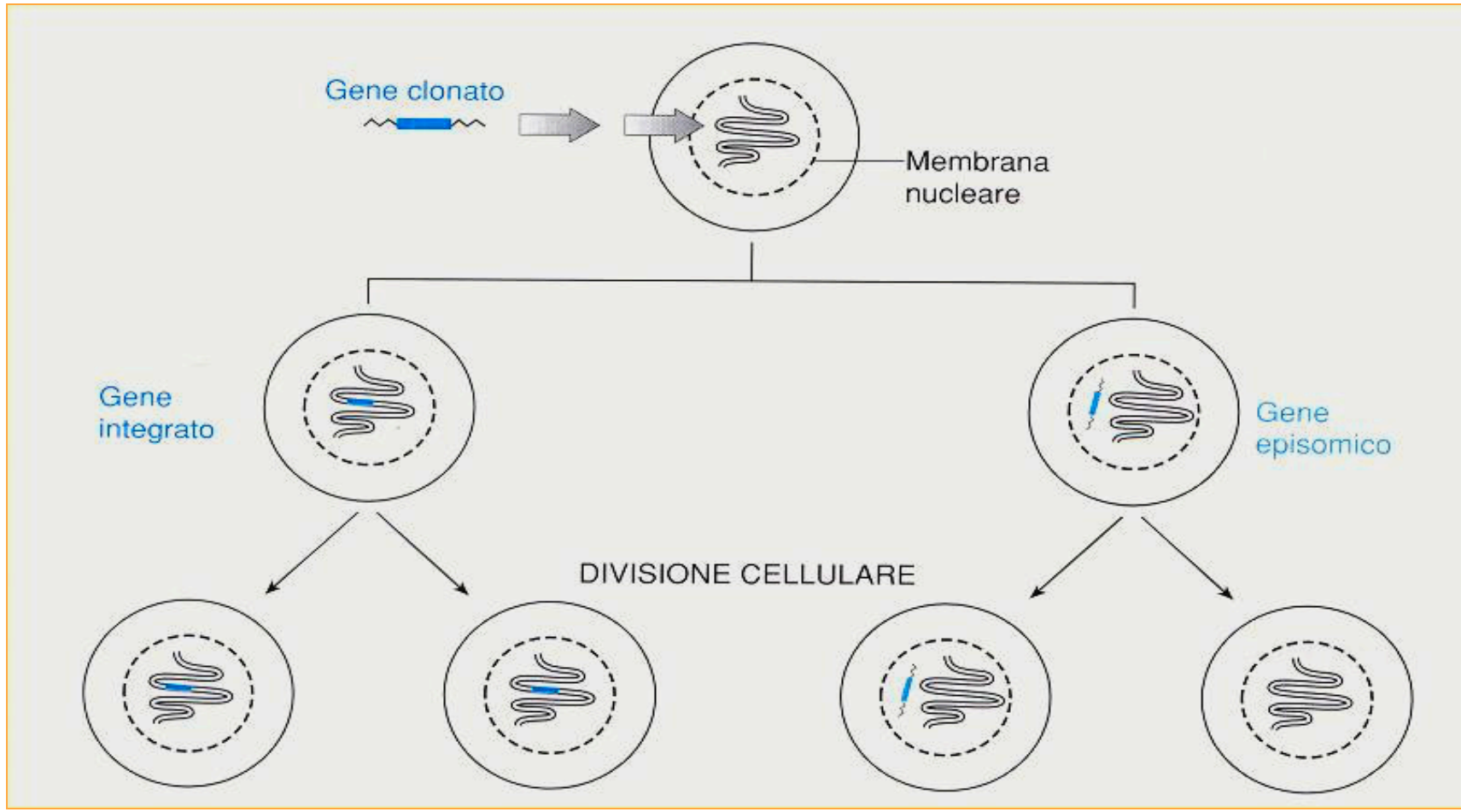
retrovirus

lentivirus



herpesvirus

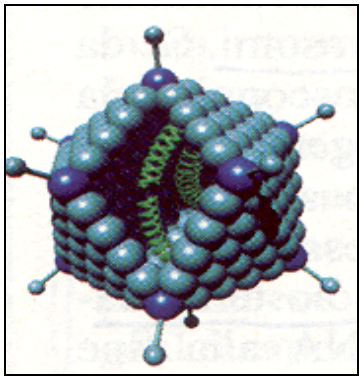
Differenze nei sistemi di rilascio



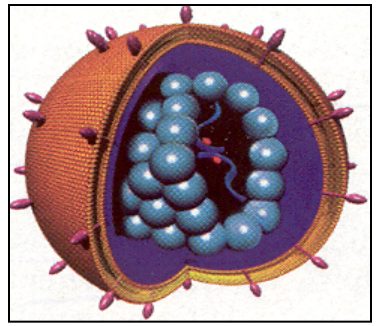
integrazione del transgene nel genoma ospita → espressione stabile

il transgene non si integra nel genoma → espressione temporanea

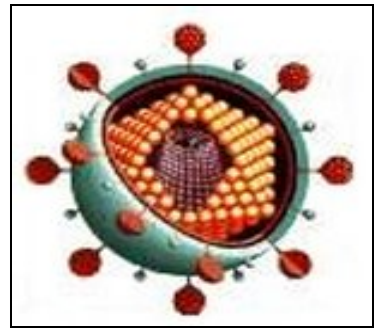
Vettori virali



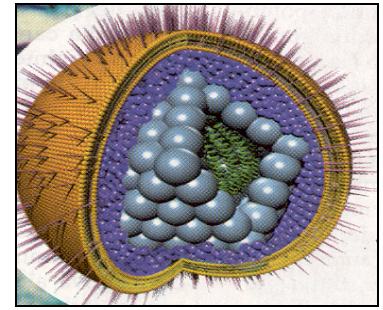
Adenovirus



Retrovirus*



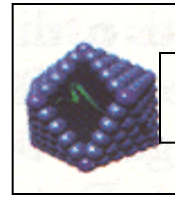
Lentivirus*



Herpes-simplex virus

Vantaggi

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



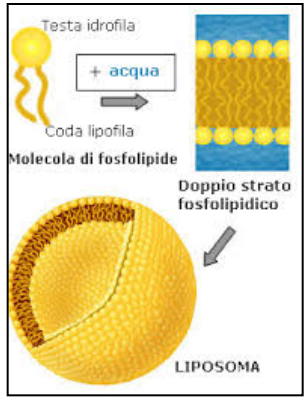
AAV*

Svantaggi

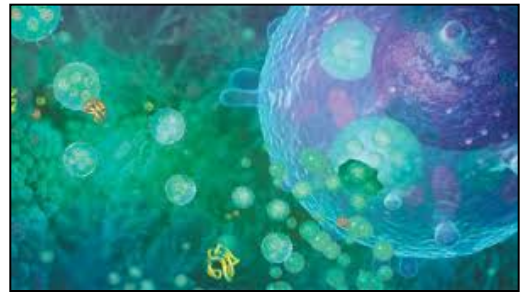
- ✓ reazione immunitaria
- ✓ tossicità
- ✓ integrazione random/mutagenesi inserzionale*

Vettori non- virali

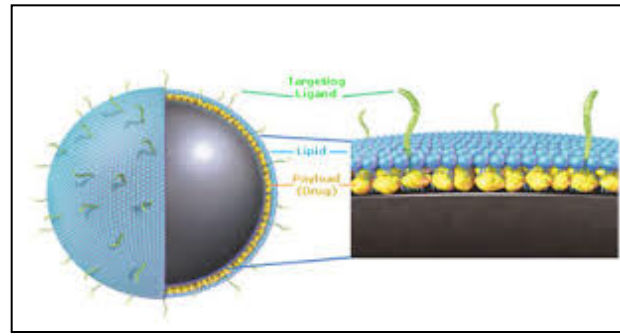
nanoparticlelle



liposomi



esosomi



nanodroplets

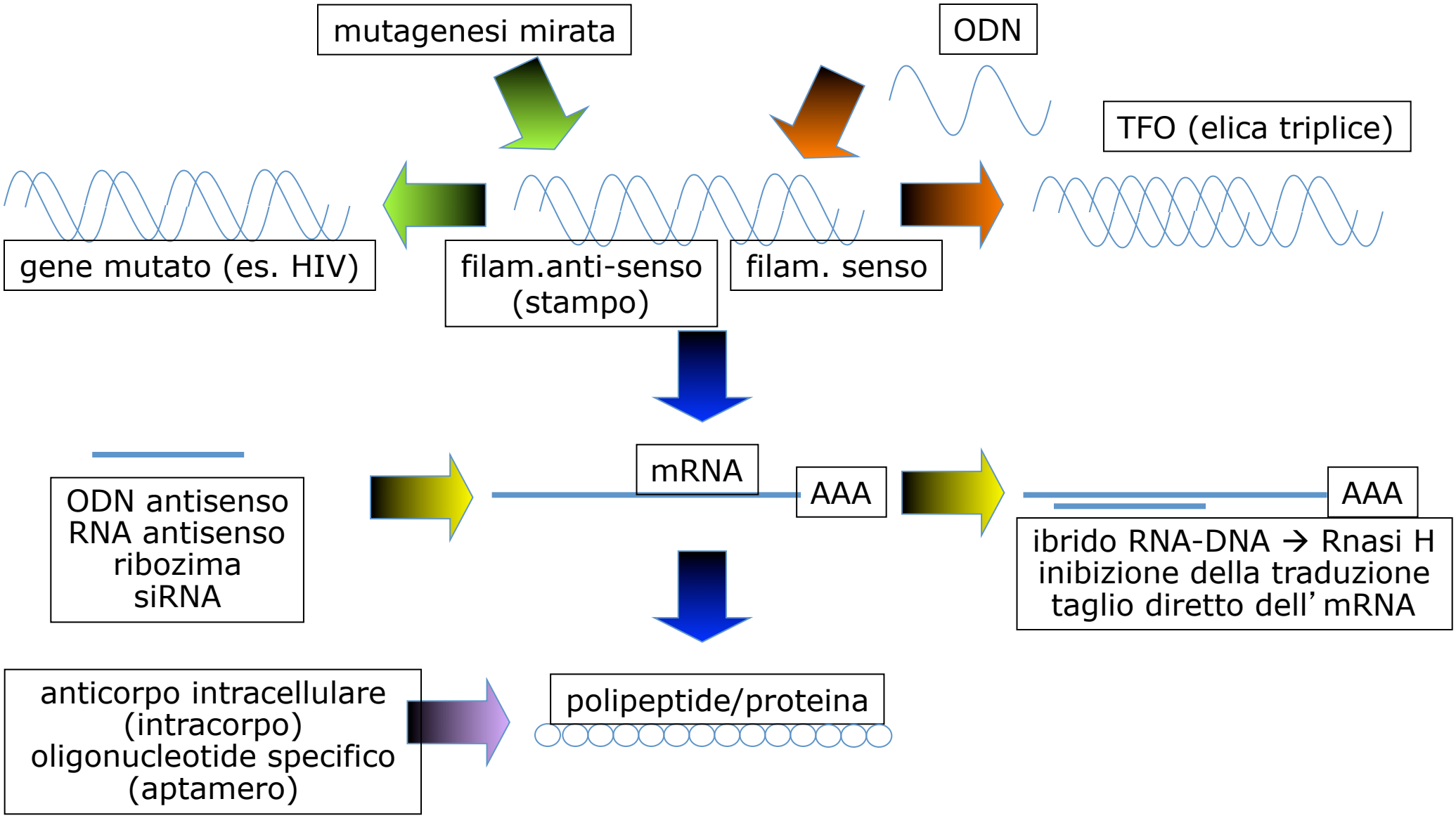
Vantaggi

- ✓ altamente efficienti nel trasporto genico
- ✓ espressione a lungo-termine
- ✓Non tossici
- ✓biodegradabili

Svantaggi

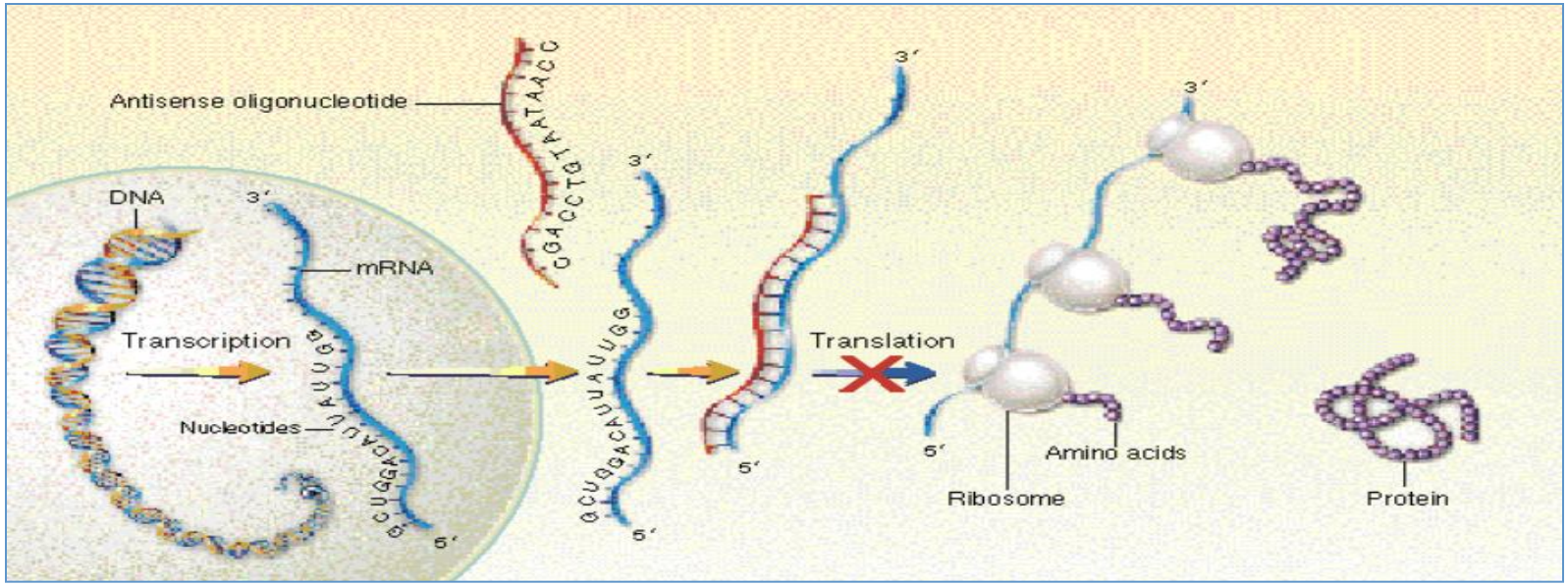
- ✓Effetti on target

Inattivazione o silenziamento Genico

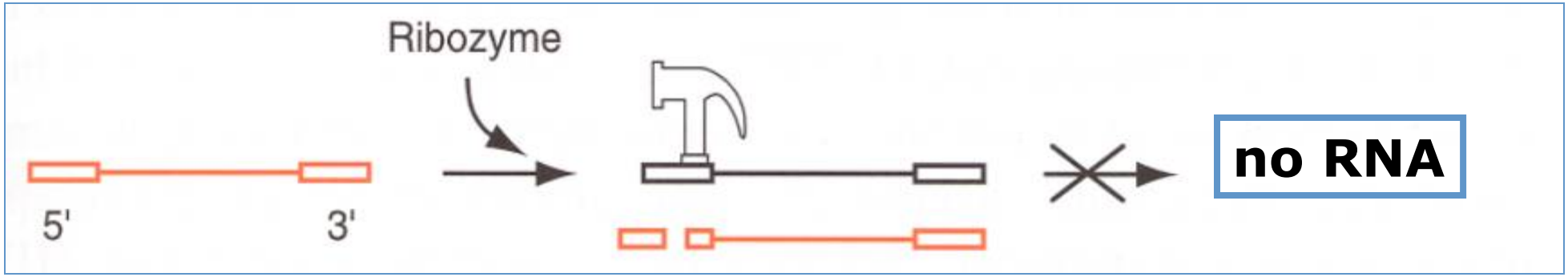


Inattivazione Genica: RNA

1. Oligonucleotide antisenso

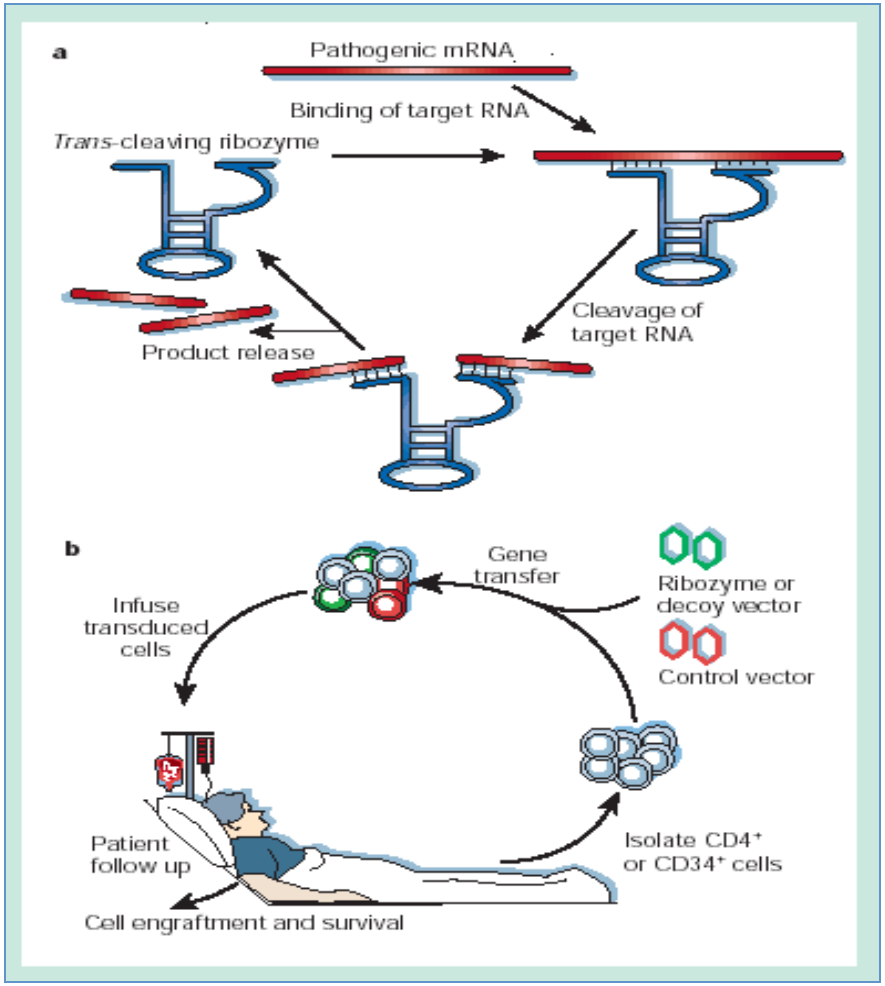


2. Ribozimi



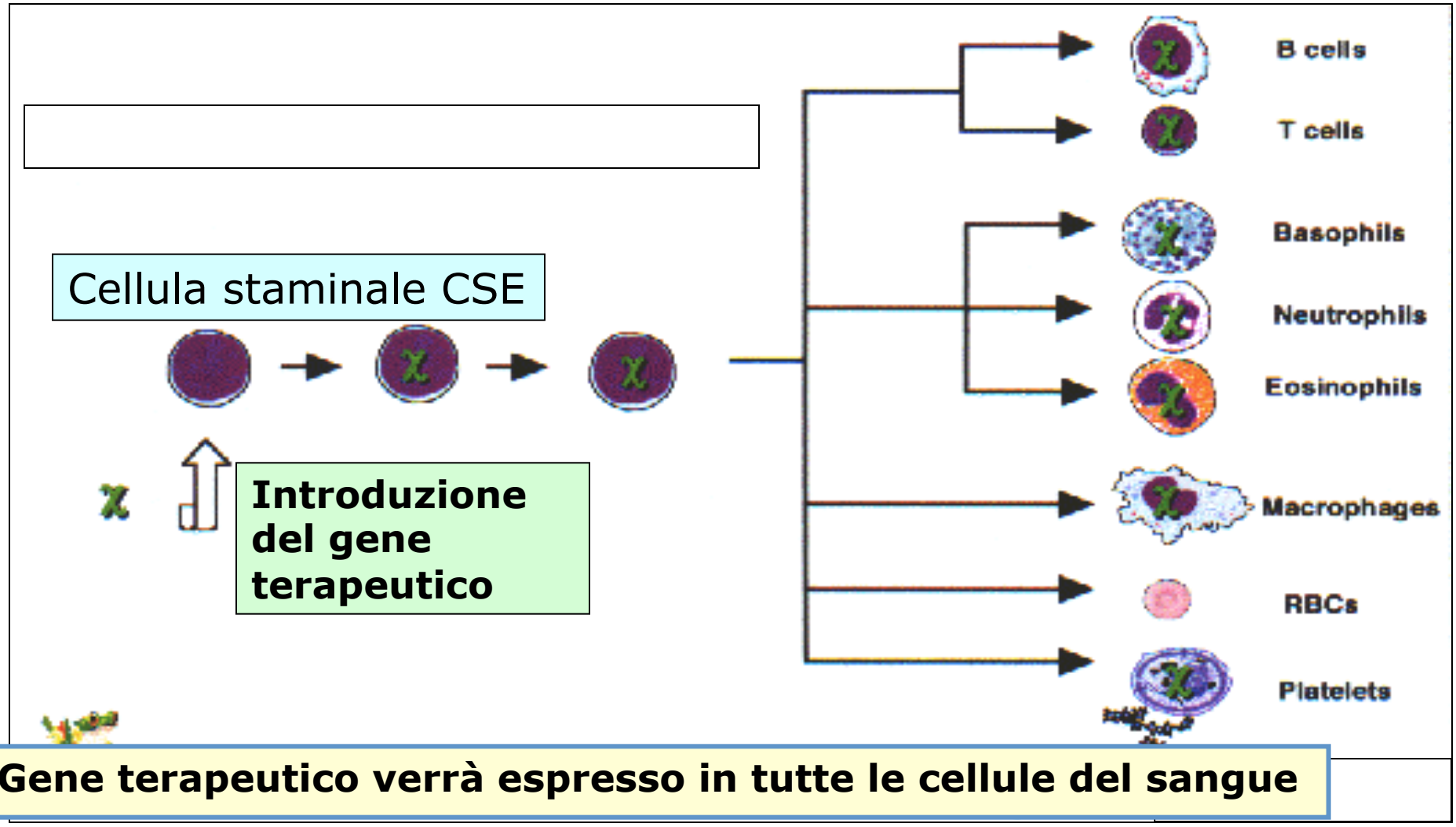
Inattivazione genica: RNA

3. RNA interference



- ✓ Interferenza mediata da RNA (RNAi) è un nuovo meccanismo per il silenziamento genico specifico
- ✓ Meccanismo evolutivam conservato come linea di difesa contro virus
- ✓ Si legano all'mRNA complementare e lo inattivano in maniera specifica
- ✓ Uso di siRNAs sintetizzati chimicam come nuova metodica di RNA terapeutico

Terapia genica in Cellule Staminali



Gene terapeutico verrà espresso in tutte le cellule del sangue

Perché usare le cellule staminali ematopoietiche?

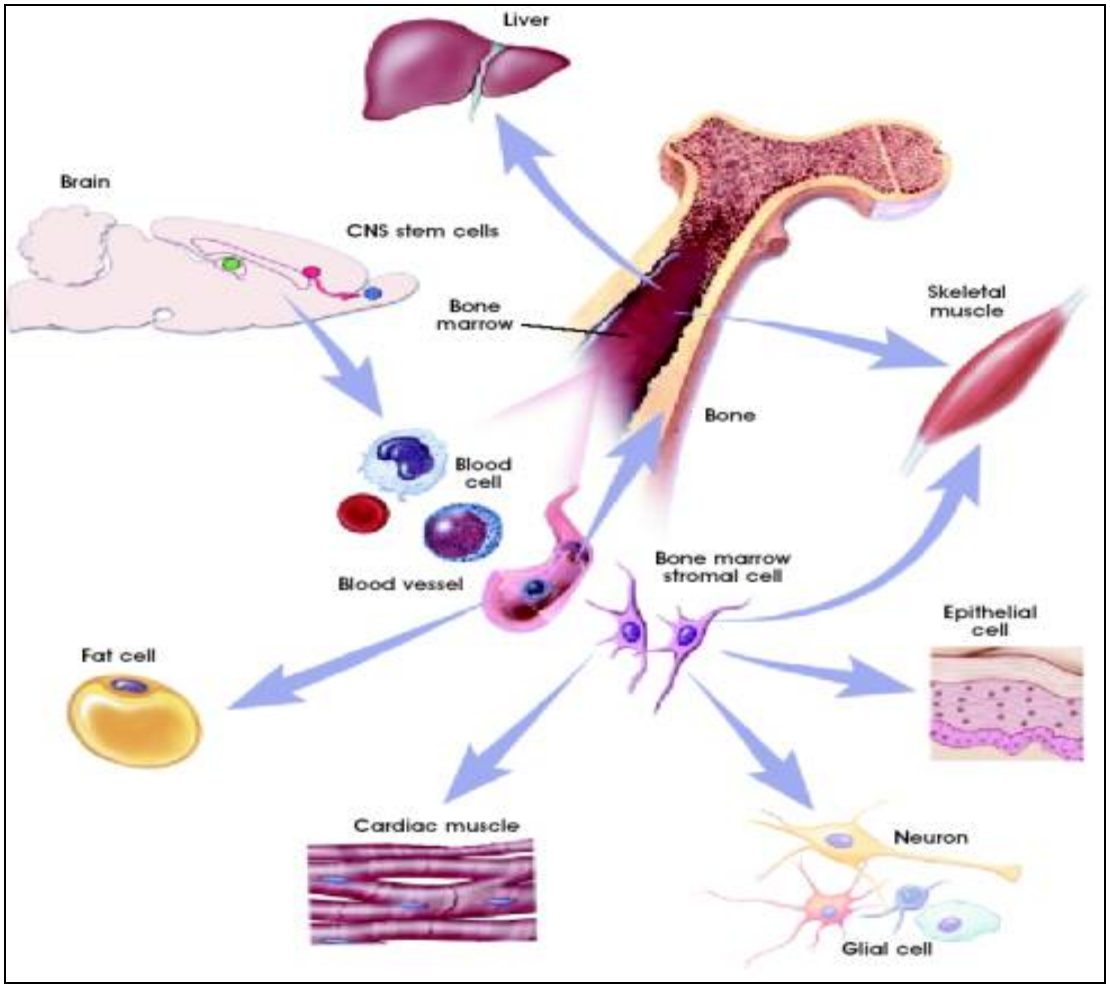
- Cellule che si autorinnovano: nessuna necessità di ripetute somministrazioni
- Poco numerose e facilmente rimovibili
- Facilmente identificabili, manipolabili e reintroducibili
- Il gene terepautico risiederà in tutte le cellule derivate
- CSE possono migrare in numerosi distretti corporei (midollo osseo, fegato, milza, linfonodi) e funzionare come vettori di trasferimento genico

Bersagli di Terapia Genica con CSE

➢ Malattie monogeniche	SCID, ADA-SCID, malattia di Gaucher, Sindrome di Hurler, anemia di Fanconi, Malattia granulomatosa cronica
➢ Trapianti e tumori	Marcatori genici, geni-suicida, geni di resistenza ai chemioterapici
➢ Immunoterapia	Vaccini a DNA/RNA, recettori chimerici cellule T
➢ Sindromi da immunodeficienza acquisita (HIV)	Riboenzimi catalitici, RNA antisenso, repressori dominanti di geni HIV, RNA decay

Terapia cellulare e genica

- ✓ Trapianti cellulari
- ✓ Trapianto cellule staminali geneticamente modificate *ex-vivo*
- ✓ Plasticità cellule staminali



SUCCESSI

- ✓ ADA-SCID, Aiuti, 2002
- ✓ X-SCID, Hacein-Bey-Abina, 2002
- ✓ Epidermolisi bullosa, De Luca, 1998
- ✓ Ferite epidermide, Quesenberry, 2002

POTENZIALITA'

- ✓ Malattie metaboliche
- ✓ Malattie ematologiche
- ✓ M. Parkinson
- ✓ M. di Alzheimer
- ✓ Infarto
- ✓ Diabete
- ✓ Artrite reumatoide

Terapia genica: risultati

- Buoni risultati in modelli animali

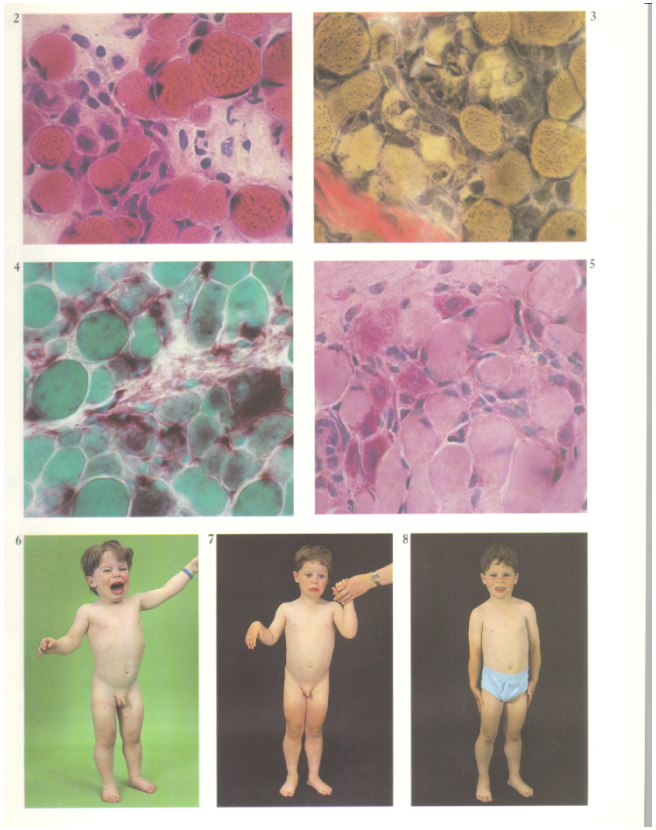
- Pochi esempi di risultati a lungo termine nell'uomo
  (X-SCID, ADA-SCID, Emofilia A)

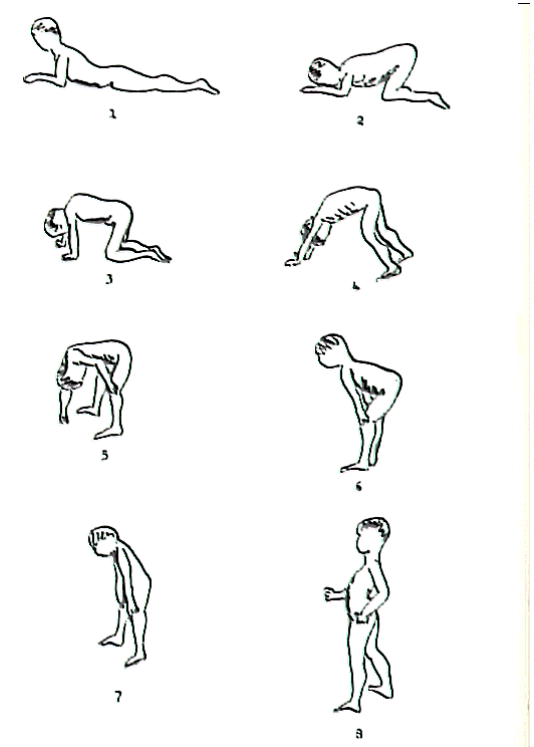
- Procedure di rilascio genico relativam sicure
  (sviluppo risposta infiammatoria sistemica, sviluppo di leucemia)

- Alcune patologie più facilmente trattabili
  (espressione non regolata, tessuti accessibili: m.metab, emofilia)

- Alcune aree più promettenti
  (vaccini a DNA, angiogenesi per m. cardiovascolari, trapianti cell)

DUCHENNE MUSCULAR DYSTROPHY





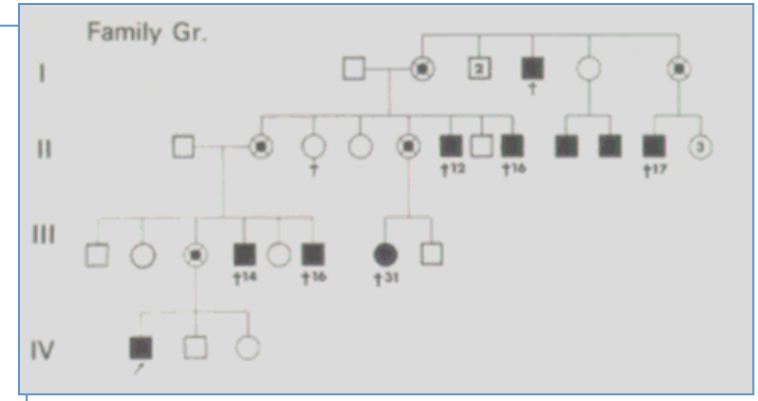
The Gower's sign

DMD

Genetic diseases inherited as a mendelian character X-linked, recessive and due to dystrophin gene mutations

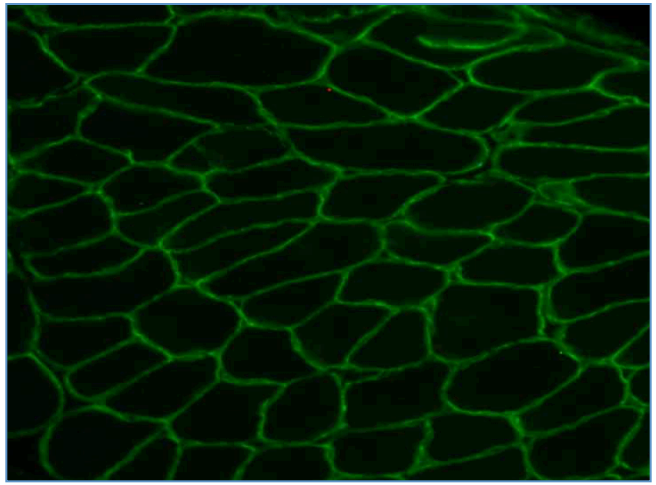
The disease affects almost exclusively males

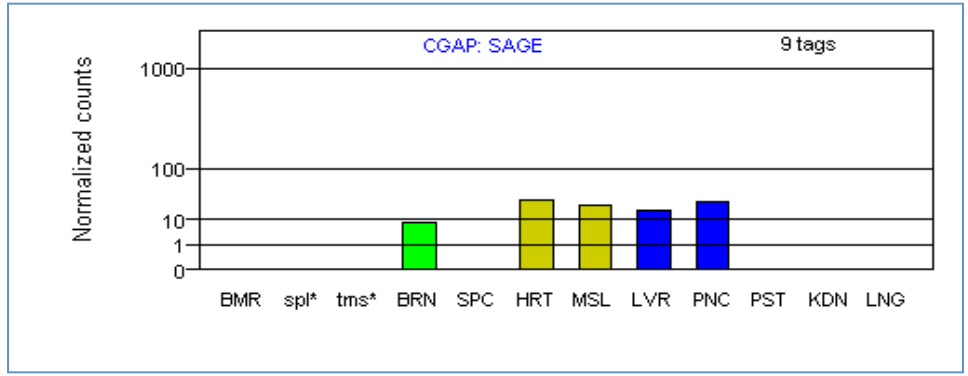
Females might be asymptomatic carriers (normal or high CK value) or mildly/severely symptomatic (skewed X-inactivation)



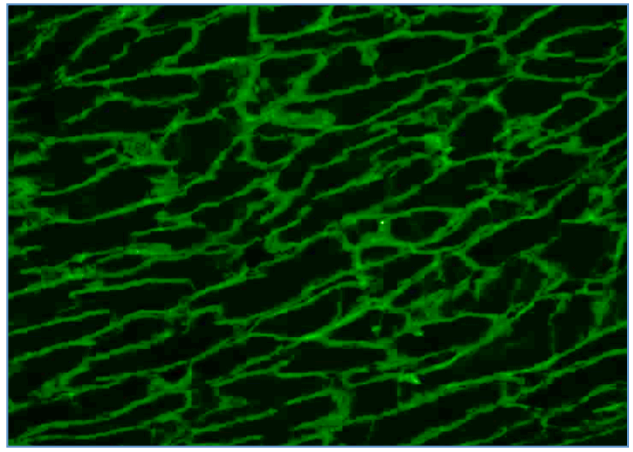
La distrofina : dove si esprime

SkM





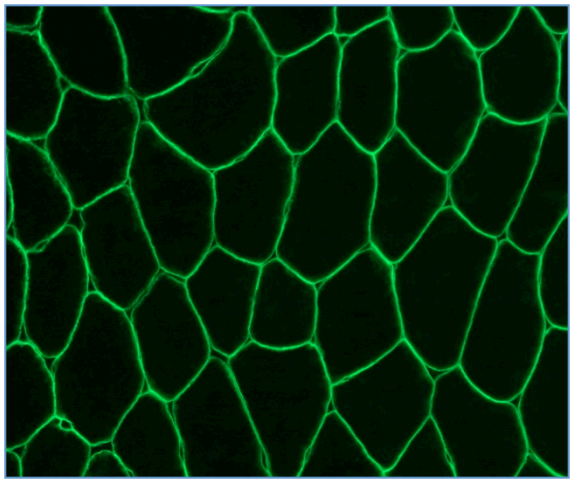
Heart



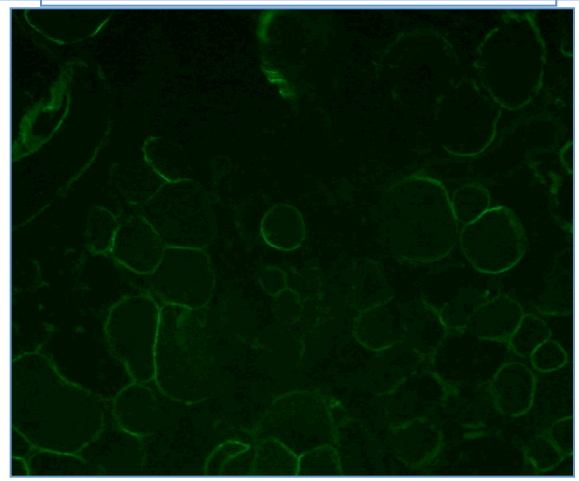
Come distinguere fra Becker e Duchenne

- **Dystrophin is virtually absent in skeletal muscle of Duchenne patients, in rare cases strongly reduced (rare revertant fibers)**
- **Dystrophin is present though qualitatively and quantitatively reduced in skeletal muscle of Becker patients**

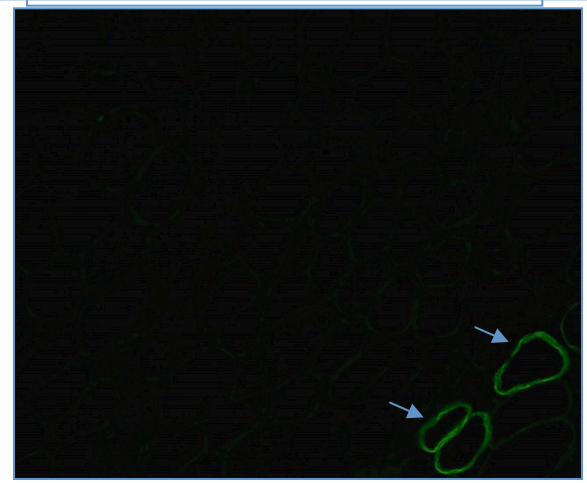
CTRL



BMD

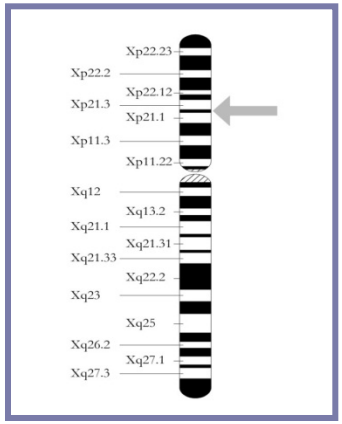


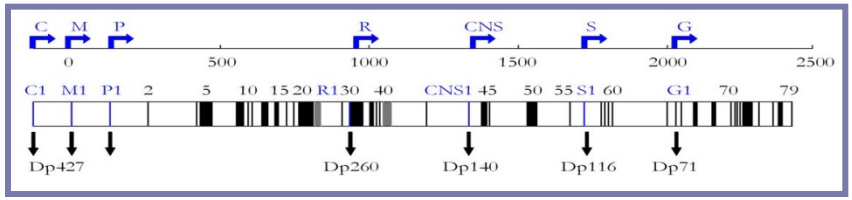
DMD

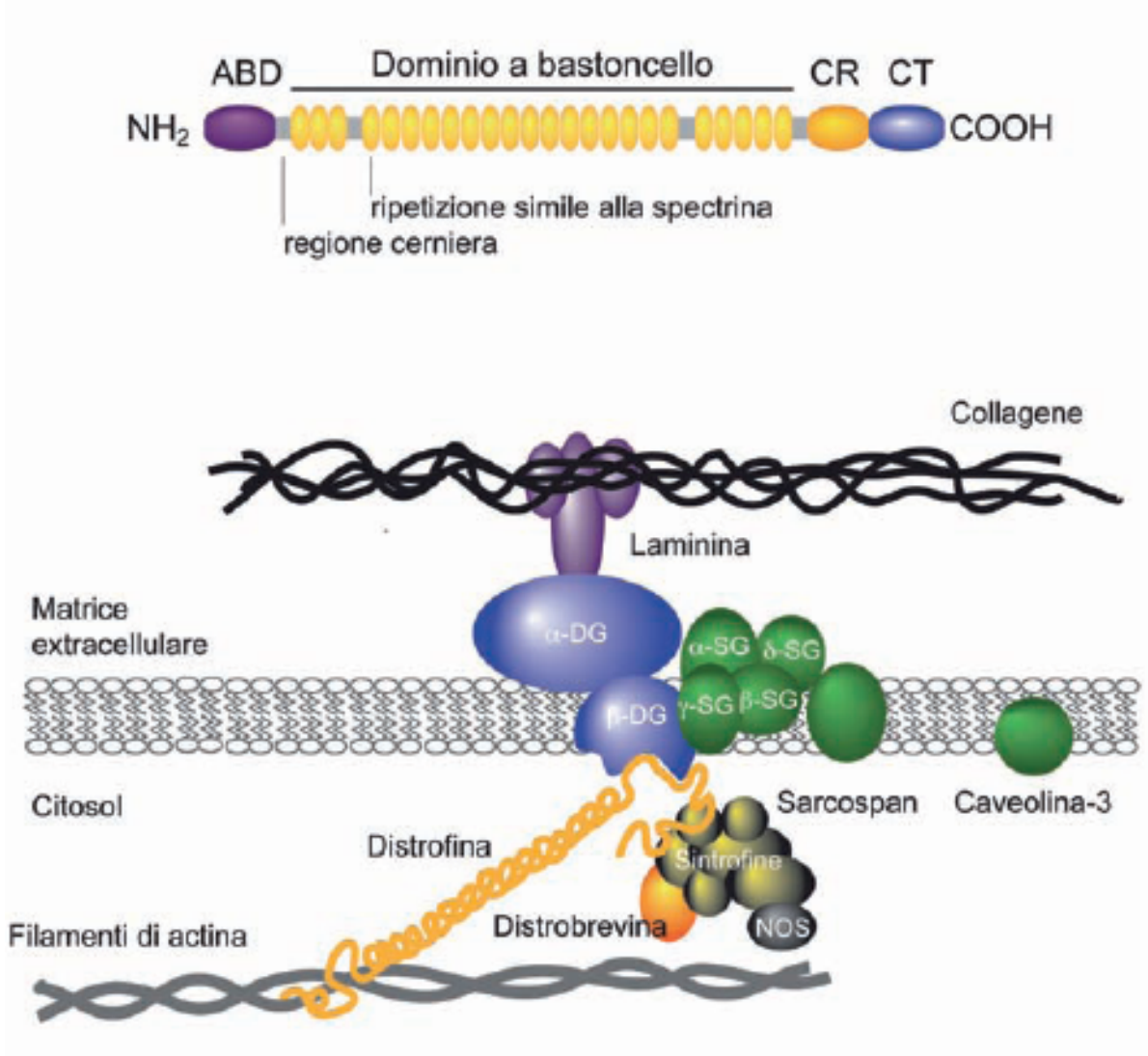


→ revertant fibers *(courtesy of Patrizia Sabatelli)*

La distrofina: il gene e la proteina



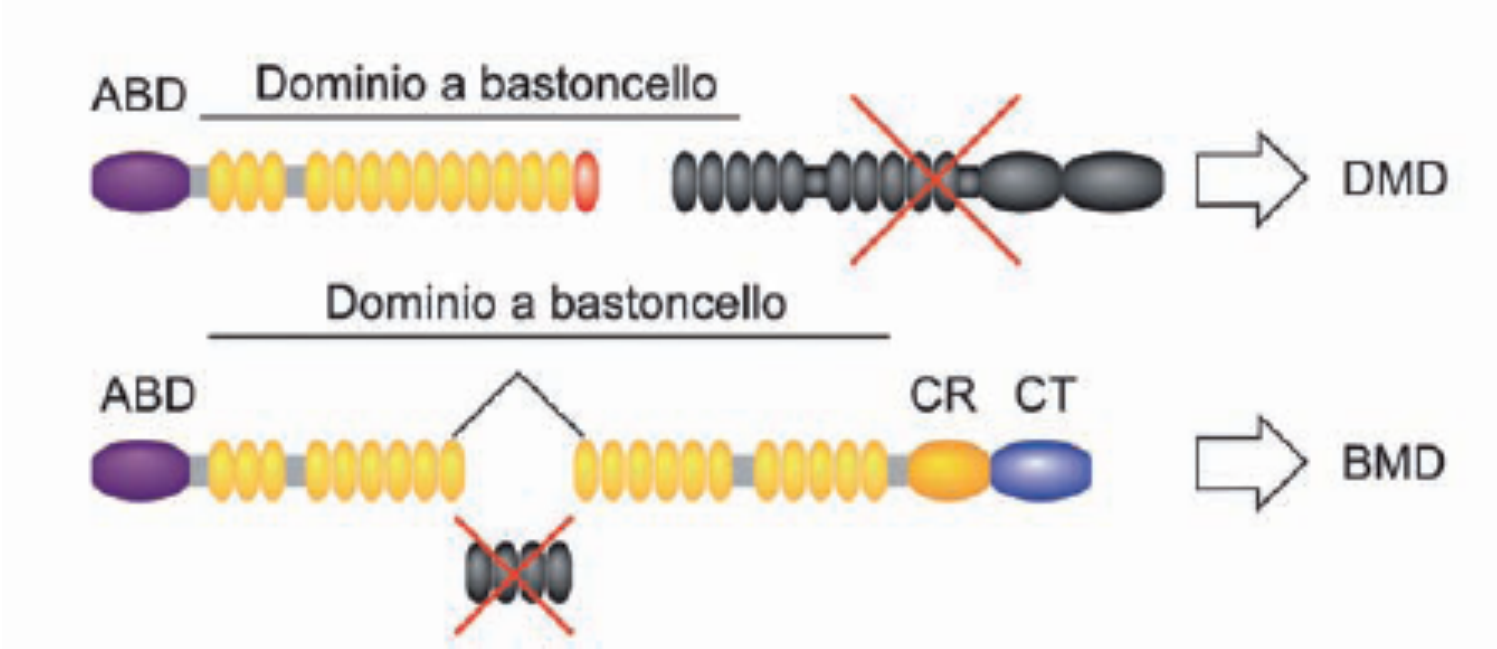




Distrofina: le mutazioni

- **65% Delezioni**
- **10% Duplicazioni**
- **22% Piccole mutazioni**
- **2% mutazioni atipiche**

Correlazioni genotipo-fenotipo:
REGOLA DEL FRAME DI MONACO



The genotype-phenotype correlation

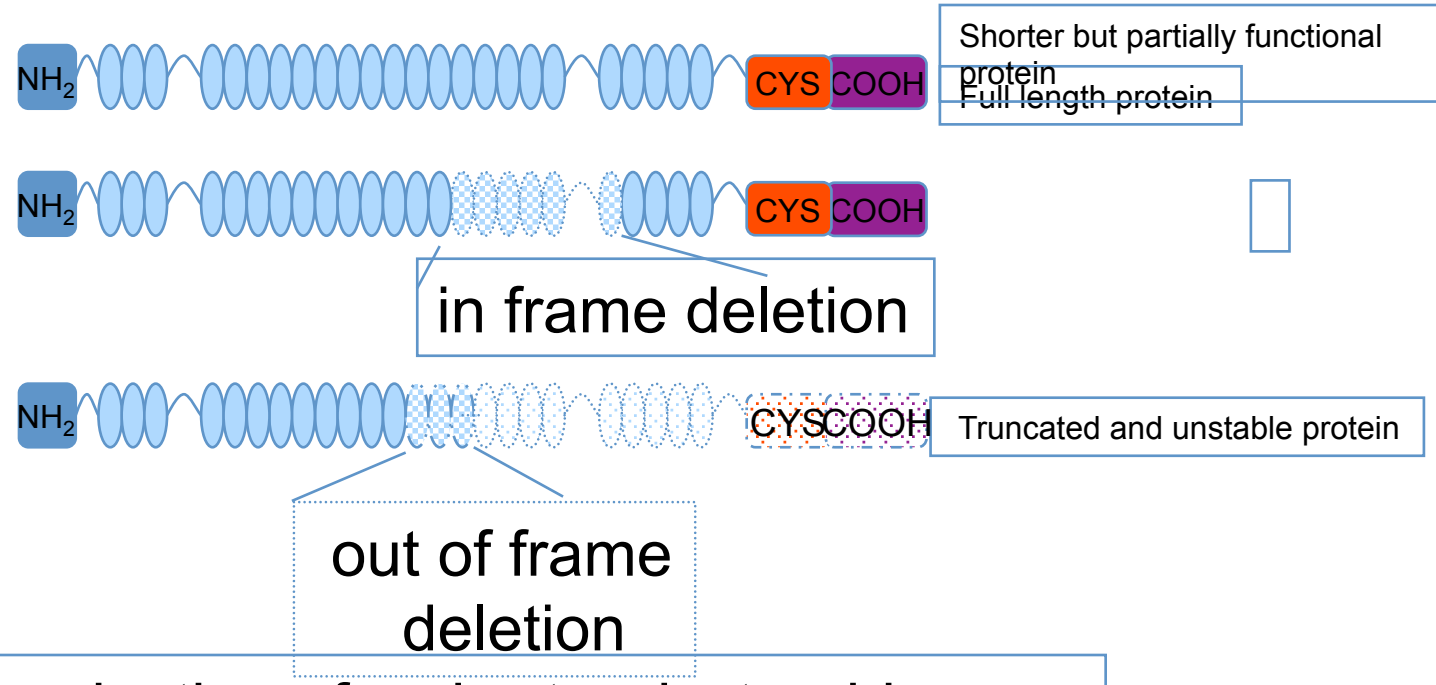
Rearrangements

DELETIONS (60%)

DUPLICATIONS (15%)

Small mutations (15-20%)

Deep intronic (1%)



Frame maintenance: production of a shorter dystrophin which determines a milder phenotype
LARGE DELETIONS DO NOT PREDICT A SEVERE PHENOTYPE
(AND VICEVERSA)

DMD scoprire la malattia VIDEO

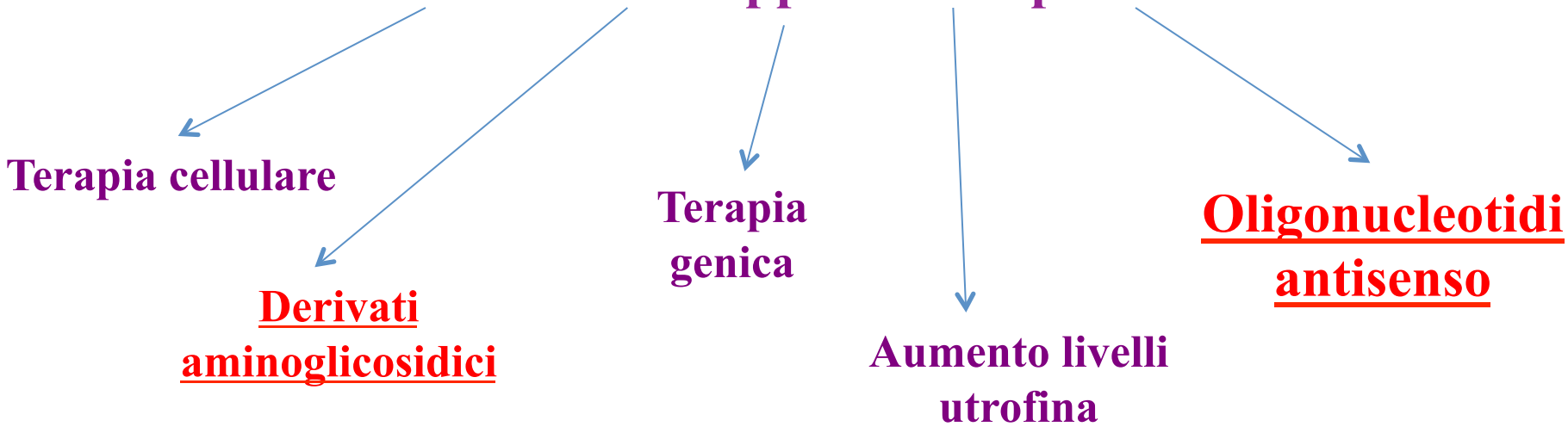
- https://www.youtube.com/watch?v=6wLnR7GJakY

VIDEO DMD IL DECORSO

- https://www.youtube.com/watch?v=AF4D4TyE9NM

Distrofinopatie: quale terapia?

- Terapie sintomatiche e di supporto
- Corticosteroidi
- Innovativi approcci terapeutici

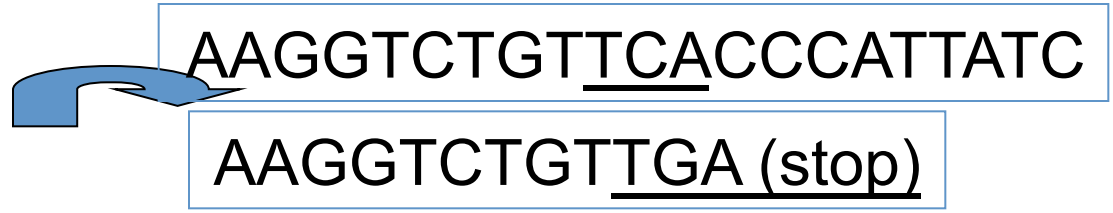


TRANSLARNA™

- **Translarna™ (ataluren) for Genetic Disorders Approved medicine in the European Union, Investigational in other jurisdictions**
- Our lead product candidate, ataluren, is a novel, orally administered small-molecule compound for the treatment of patients with genetic disorders due to a nonsense mutation. Ataluren is in clinical development for the treatment of Duchenne muscular dystrophy caused by nonsense mutations (nmDMD) and cystic fibrosis caused by nonsense mutations (nmCF). Ataluren was granted marketing authorization in the European Union under the trade name Translarna™ for the treatment of nmDMD in ambulatory patients aged five years and older. Translarna is the first treatment approved for the underlying cause of DMD. The European Medicines Agency, or EMA, has designated ataluren as an orphan medicinal product and the U.S. Food and Drug Administration, or FDA, has granted orphan drug designation to ataluren for the treatment of both nmDMD and nmCF.

PTC124 Stop mutations correction

- Stop (nonsense)

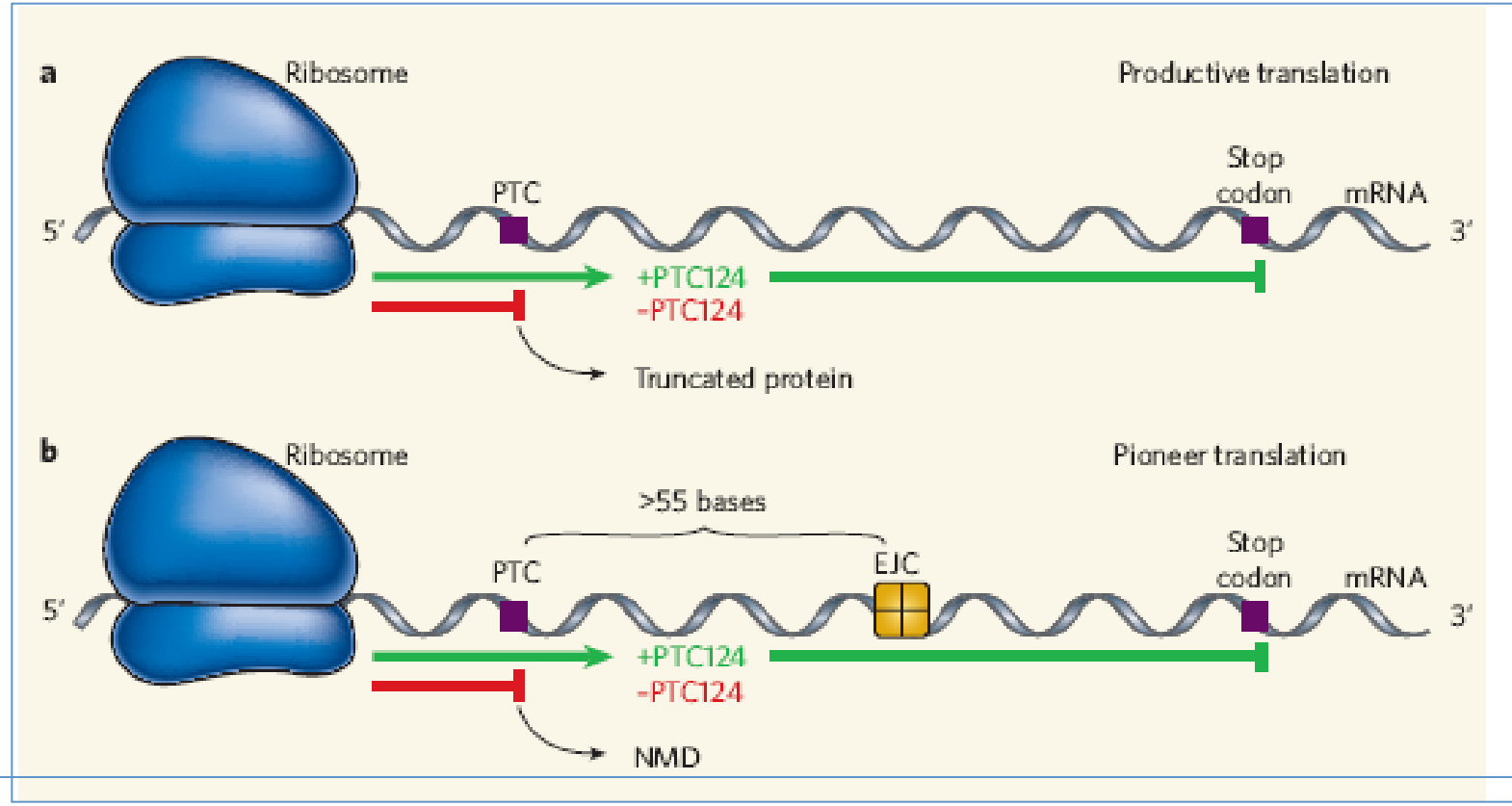


Lead to TRUNCATED non functional proteins
(generally DMD phenotype)

PTC124: drug able to convert a PREMATURE STOP CODON (TGA, TAA, TAG) in a SENSE codon.

Ribosomal correction (post-transcriptional)

PTC124 (gentamicin analogue): read through premature termination codons

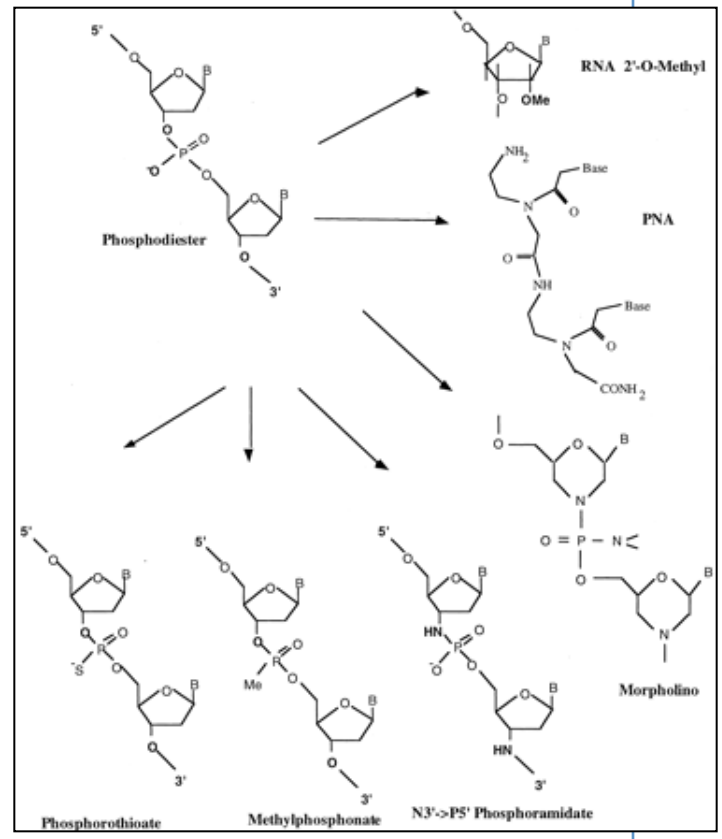


PTC124: it may correct nonsense mutations only!

video

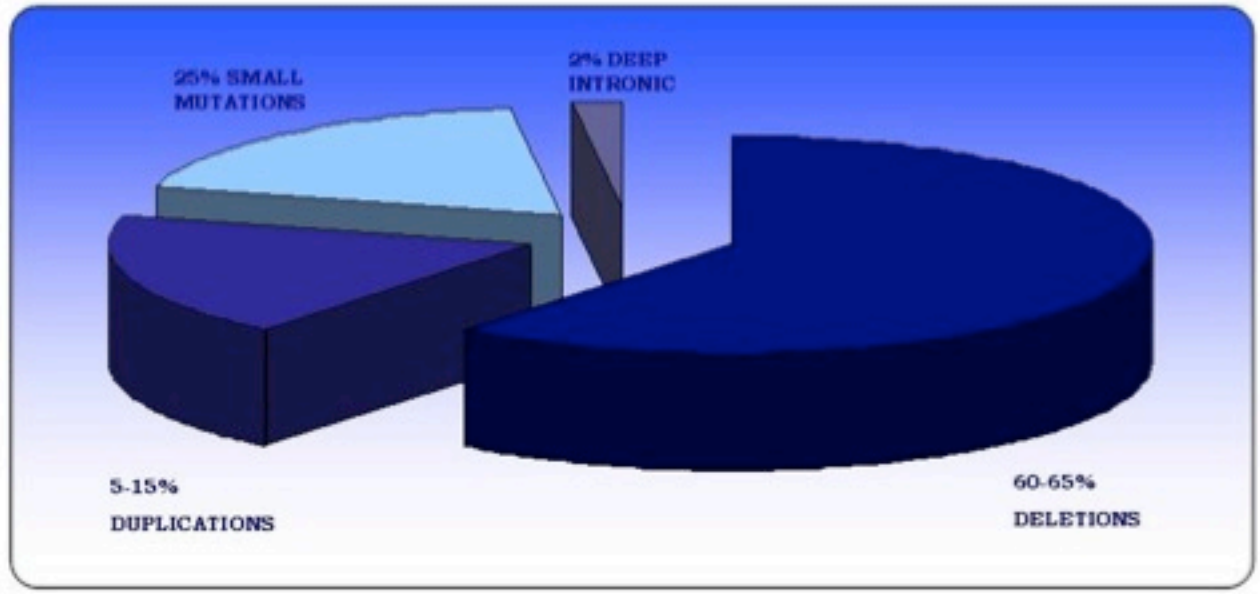
- https://www.youtube.com/watch?v=THJJCMbjGf0

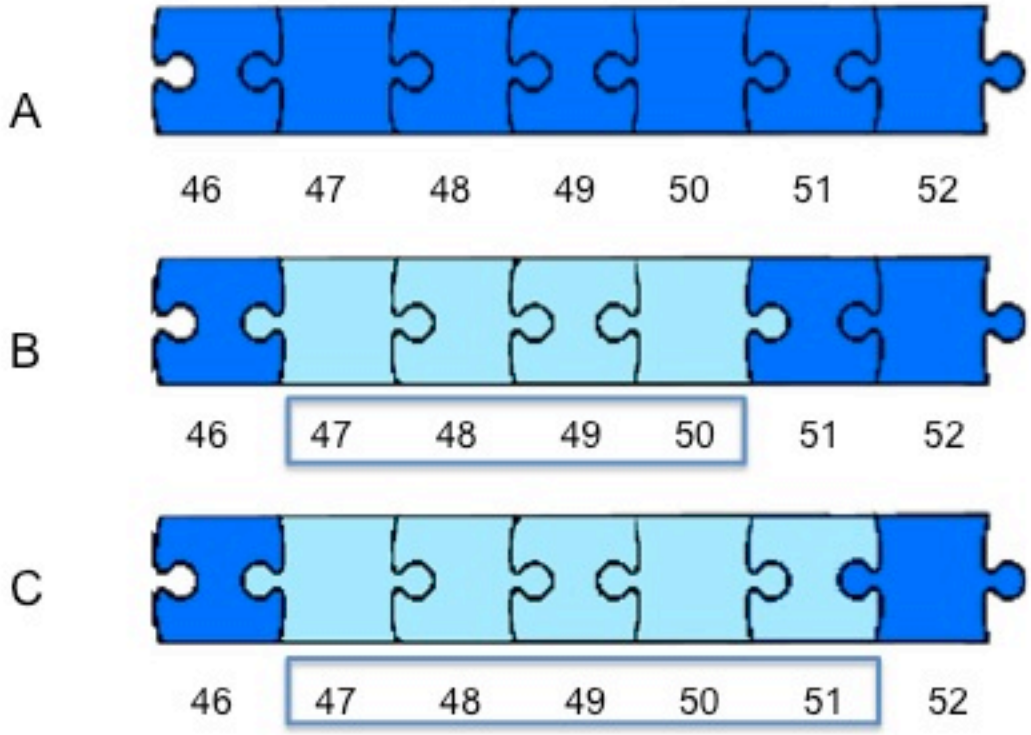
Come funziona la terapia molecolare tramite Exon-SKipping



L' AON riconosce uno specifico ESONE (che fiancheggia gli esoni deleti) e induce la OMISSIONE ESONICA (exon skipping) dell' esone.

Tale omissione ripristina la cornice di lettura (frame) dell distrofina e consente la produzione di una proteina più corta ma funzionante


25% SMALL MUTATIONS
2% DEEP INTRONIC
5-15% DUPLICATIONS
60-65% DELETIONS


A
46 47 48 49 50 51 52
B
46 47 48 49 50 51 52
C
46 47 48 49 50 51 52

Restoring the frame: AON drugs

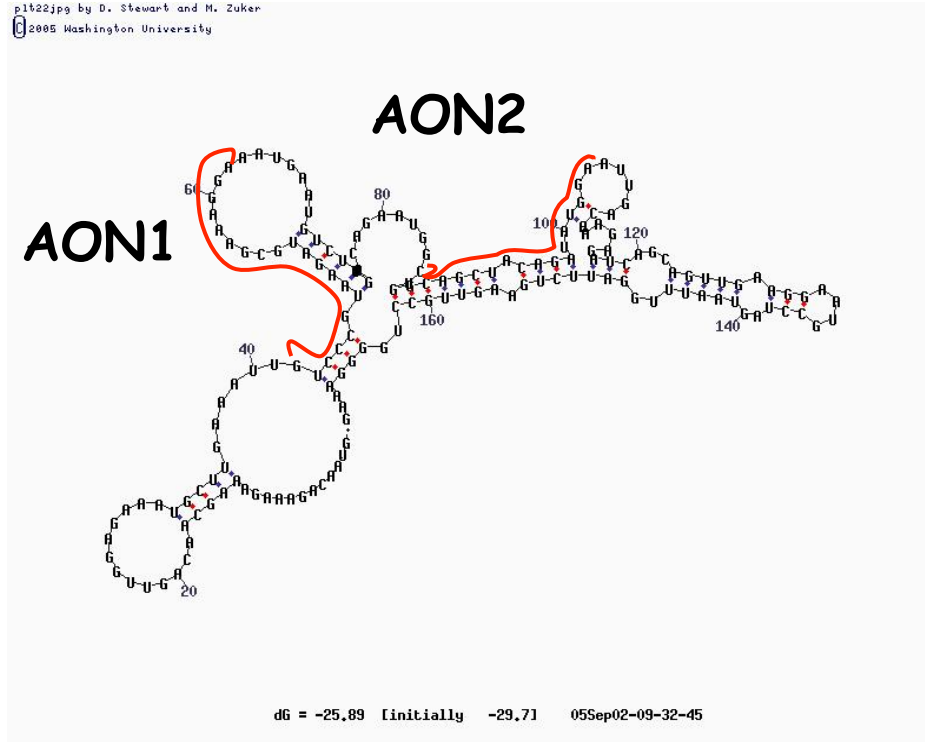
AON

Sono Farmaci orfani (Orphan Drug designation EMA)

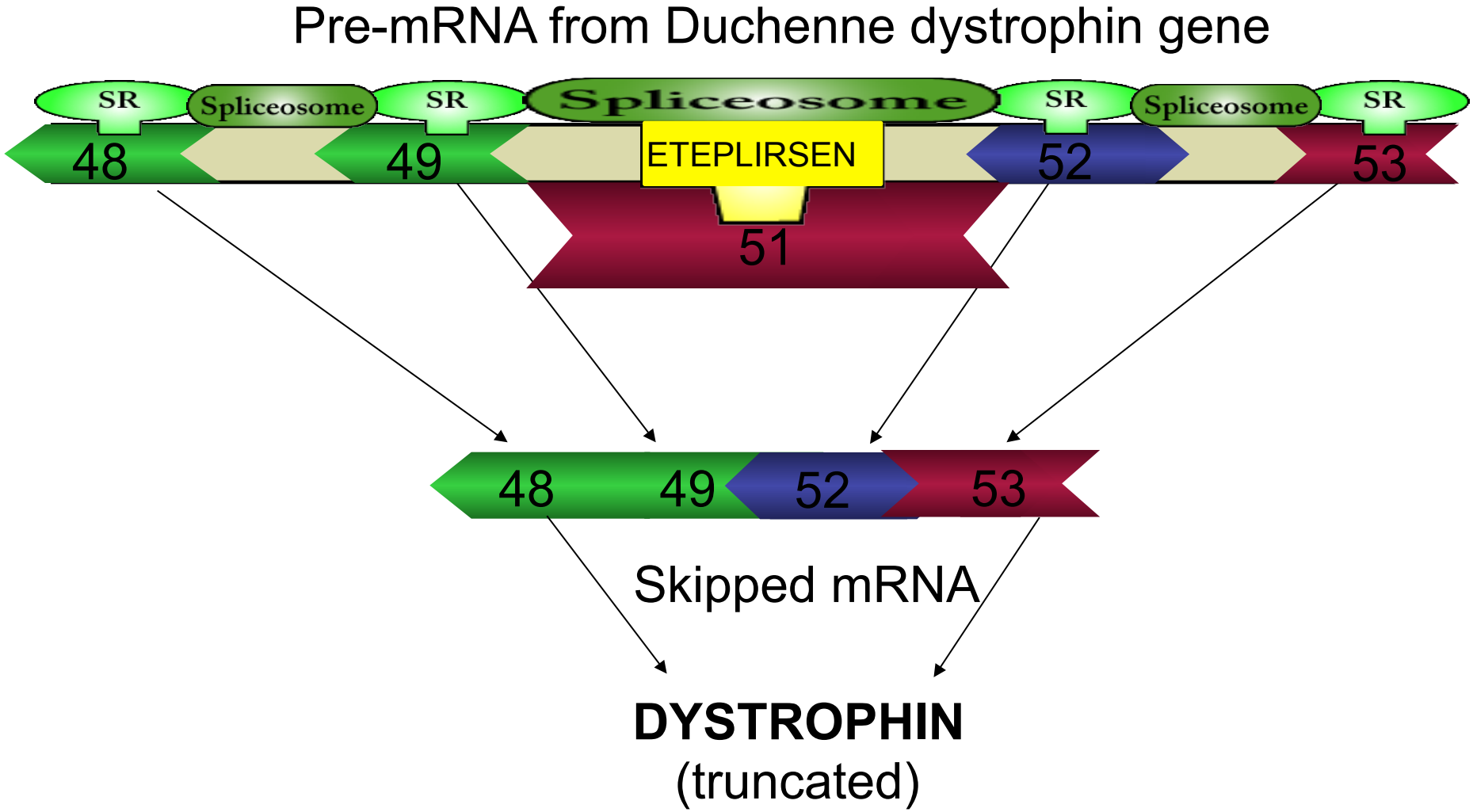
Composizione:

RNA (20-23 mer) con «ossatura» di gruppi fosforotioati o morfolinici

(PMO or 2'OMePS)



EXONDYS51 ETEPLIRSEN



Terapia con Antisenso

VANTAGGI

Trial sistemici (somministrazione sottocutanea o intravenosa))

No risposta immunitaria

No immunosoppressione

No integrazione/ricombinazione e effetti genotossici

Trattamenti reversibili

SVANTAGGI

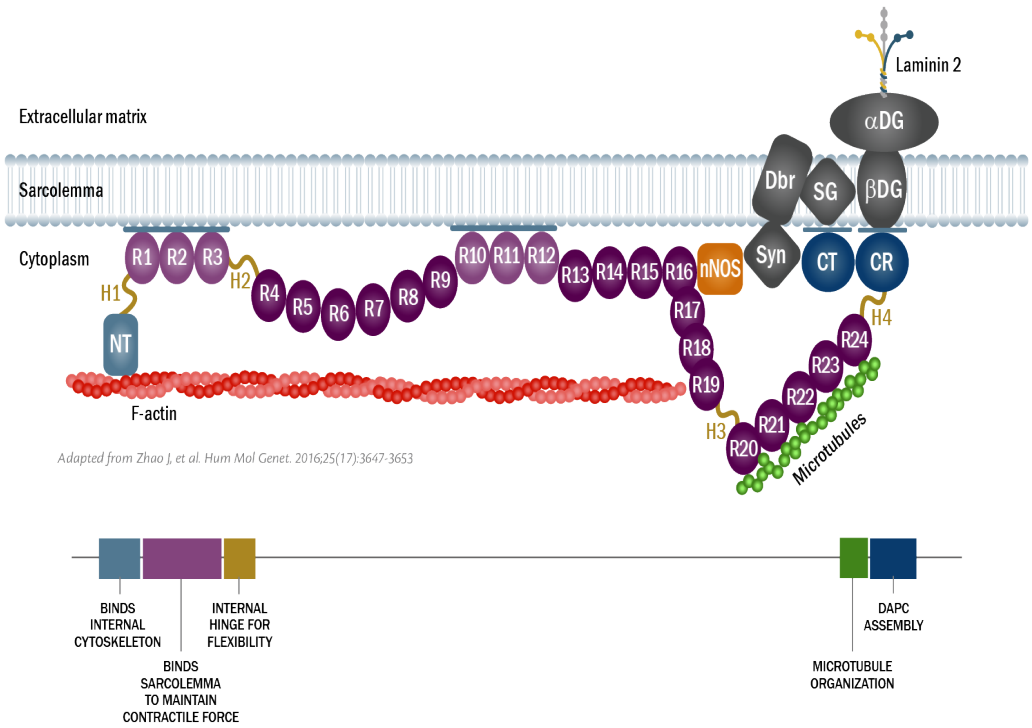
Terapie croniche

Efficienza media

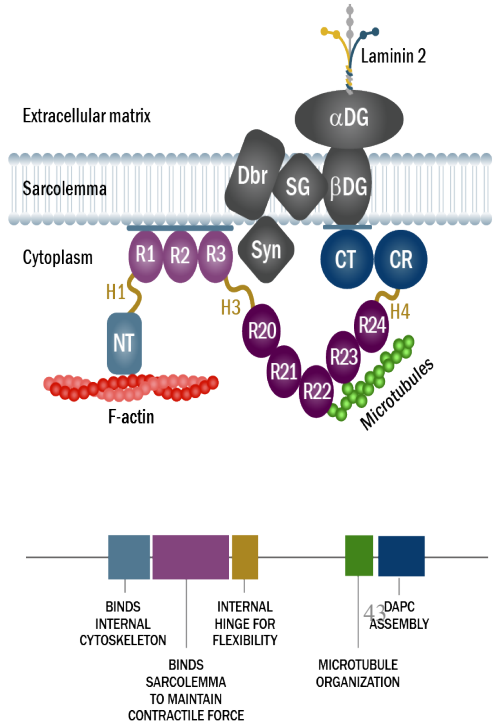
Mutazione specifici (*Personalised Medicine*)

terapia genica con microdistrofina

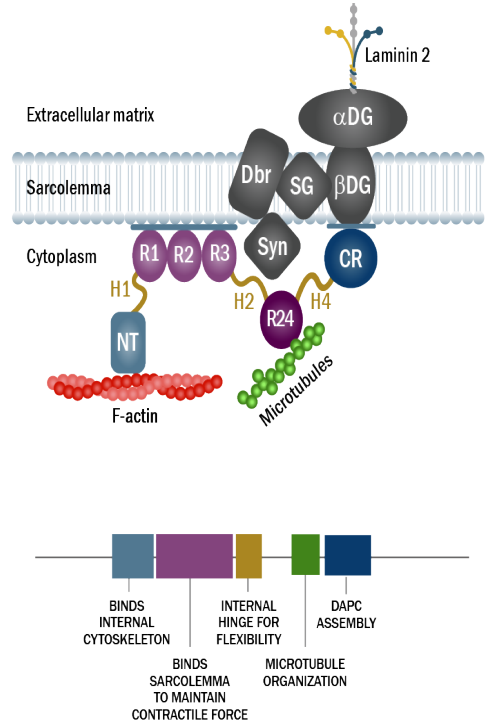
NORMAL MUSCLE[1-5]



MILD BMD (61 YoA ambulatory)[6-7]



SAREPTA MICRO-DYSTROPHIN[1-5]



Dbr, dystrobrevin; DG, dystroglycan; f-actin; filamentous; nNOS, neuronal nitric oxide synthase; NT, amino terminal; SG, sarcoglycan; Syn, syntrophin.

1. Gao Q *et al*. *Compr Physiol.* 2015 July 1; 5(3):1223. 2. Harper SQ *et al. Nature Med.* 2002 March; 8(3):253. 3. Nelson DM *et al. Human Molec Genetics.* 2018 27(12):2090. 4. Fairclough RJ, *et al*. *Nat Rev Genet* 2013;14:373-8. 5 Aartsma-Rus, A., et al., *Muscle Nerve*, 2006. 34(2): p. 135-44. 6. England SB, et al. *Nature*. 1990;343(6254):180-182. 7. Wells DJ, et al. *Hum Mol Genet*. 1995;4(8):1245-1250.

Characteristics of adeno-associated virus

- Adeno-Associated Virus

Not associated with any disease state

Very rarely integrates

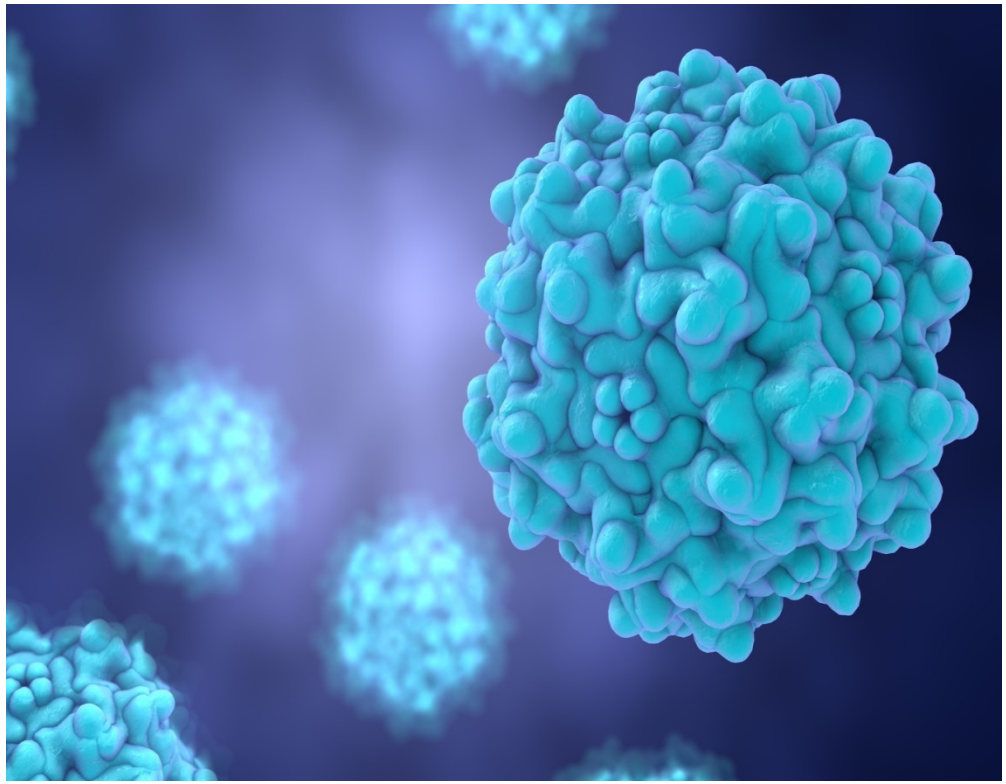
Small (20–25 nm) replication-defective, non-enveloped virus

Small packaging capacity (~4.7 kb)

Able to infect dividing and nondividing cells

Preexisting humoral and cellular immunity to many common wild-type serotypes

Graphical representation of AAV

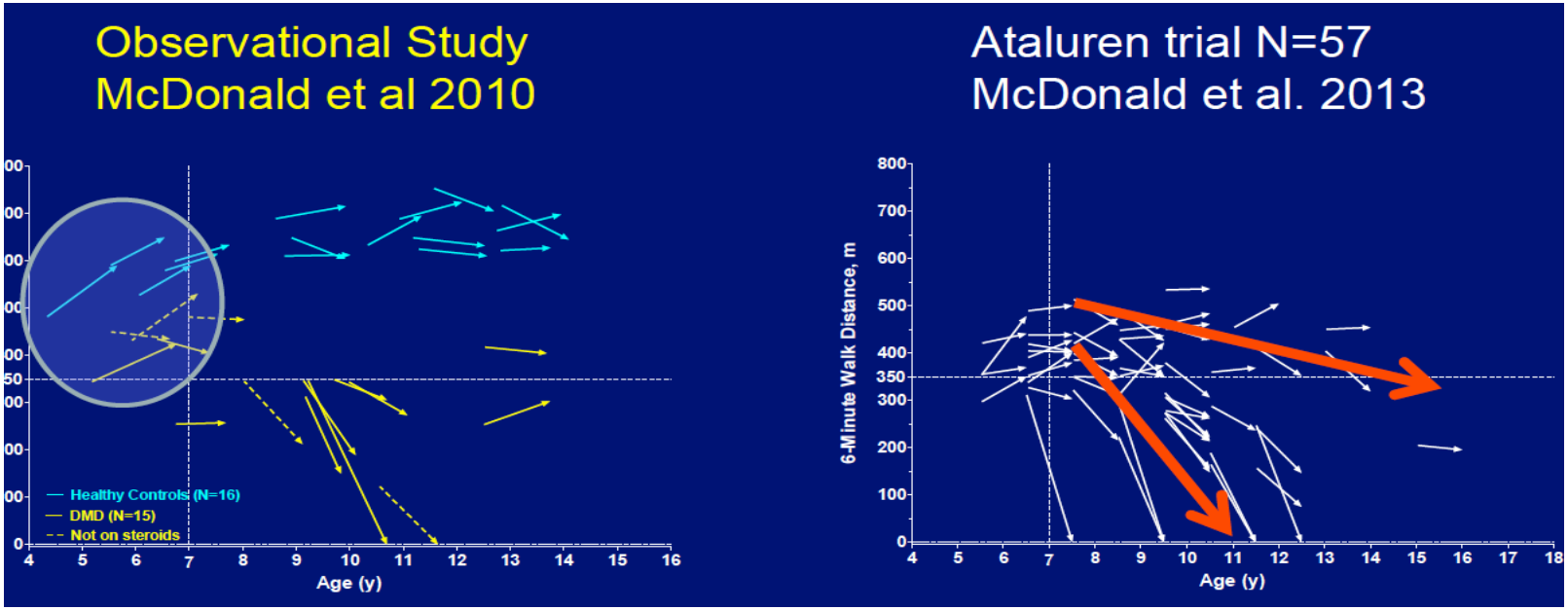


Obiettivi DEGLI STUDI CLINICI

- Efficacia: espressione di distrofina
  funzione muscolare
  forza muscolare
- Sicurezza: ECG, Parametri vitali
  parametri biochimici
  parametri ematologici
  parametri di coagulazione
  marcatori infiammazione
  cistatina C
  analisi urinarie
- Tollerabilità: reazioni avverse sistemiche e/o locali

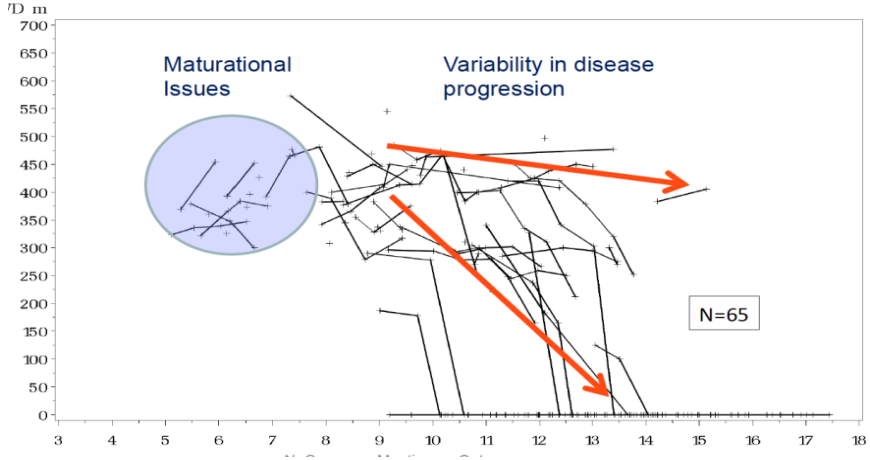
Prove di efficacia

- 6MWD test
- 10MW test
- 4Stair Climb
- Rise from floor (Gower's)
- Myometry
- Spirometry

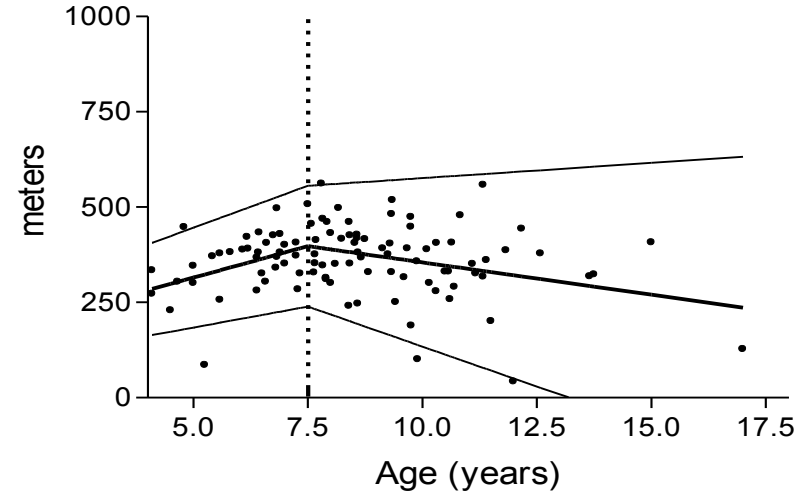


NATURAL HISTORY DATA In DMD

Goemans et al, 2013

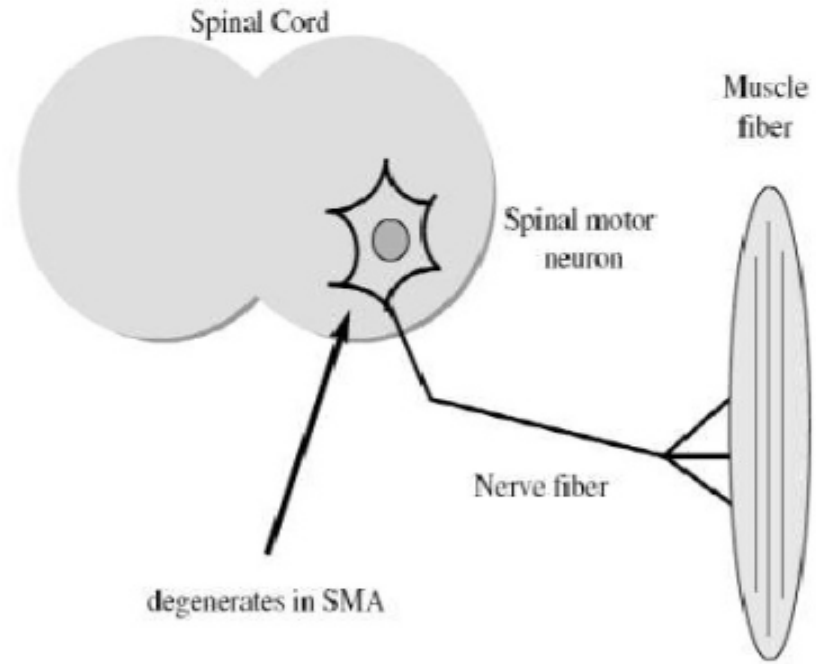


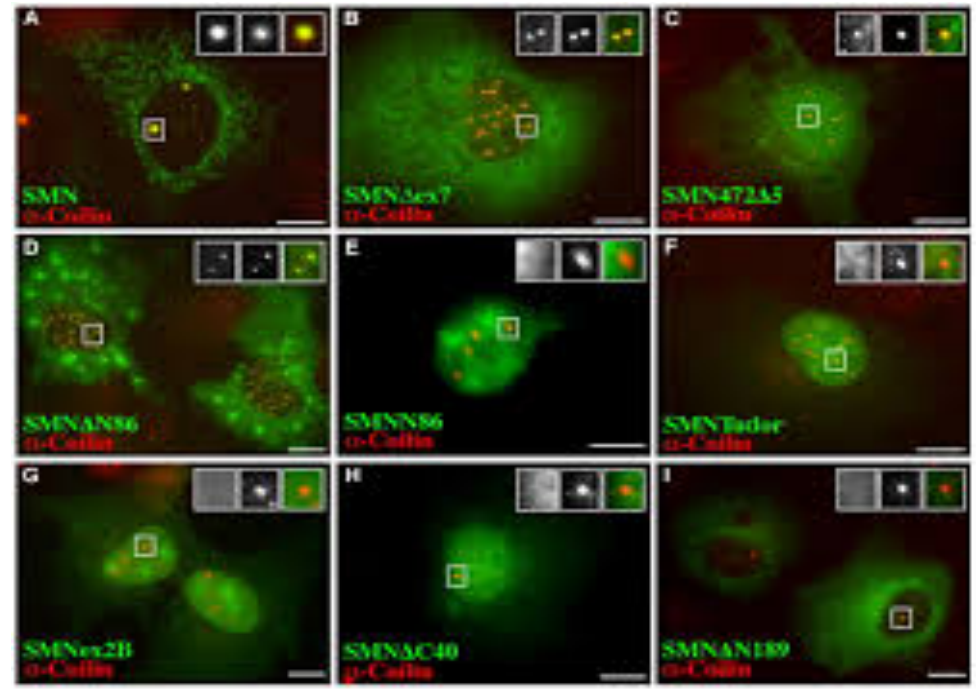
Italian network Mazzone 2012, 2013



SMA –spinal muscular atrophy

- The spinal muscular atrophies (SMAs) are characterized by degeneration of the anterior horn cells in the spinal cord and motor nuclei in the lower brainstem.





SMN protein

Expressed in most tissues

High levels are found in spinal motor neuron

SMN exist in the cell as a part of a large complex that regulates the assembly of a specific class of RNA protein complexes - which is essential for pre-mRNA splicing.

The function of SMN protein is linked to the control of protein synthesis.

PHENOTYPE CLASSIFICATION

- SMA TYPE 0 (congenital)
- SMA TYPE I (Werdnig-Hoffmann)
- SMA TYPE II (Werdnig-Hoffmann late)
- SMA TYPE III (Juvenile)
- SMA TYPE IV (Kugelberg-Welander)

SMA TYPE I-II

- Severe form of SMA
- Onset : first 6 months
- Death : < 2 year
- **Never raising the head or sitting**

SMA TYPE III

- Less sever
- Clinical appearing : < 18 months
- **Able to sit unaid**
- Death : about 9 years

SMA TYPE IV

- Mildest form of SMA
- Onset : > 18 months
- **Walking without aid**

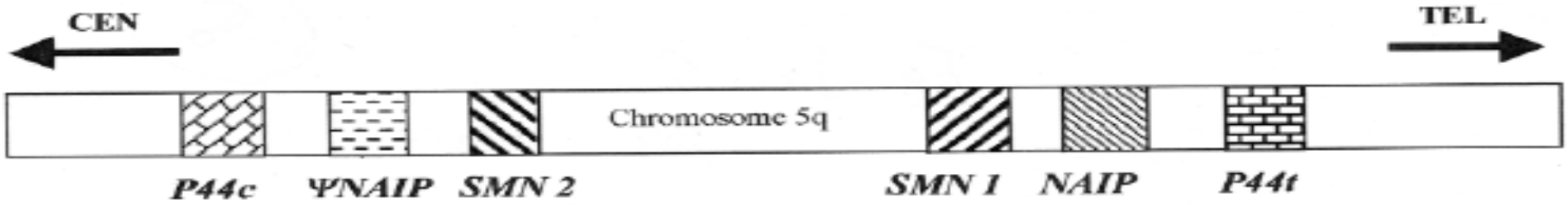
SMA THE CLINICS

https://www.youtube.com/watch?v=dUAbRXkWeuo

GENETICS

- 1990: The three types of SMA were mapped to 5q13
- The SMA locus contains two inverted copies of a 500kb element
- The two copies are named telomeric (SMN1) and centromeric (SMN2)

GENETIC MAP



- Three candidate genes named SMN (Survival Motor Neuron), NAIP (Neuronal Apoptosis Inhibitory Protein) and P44 were identified in this locus

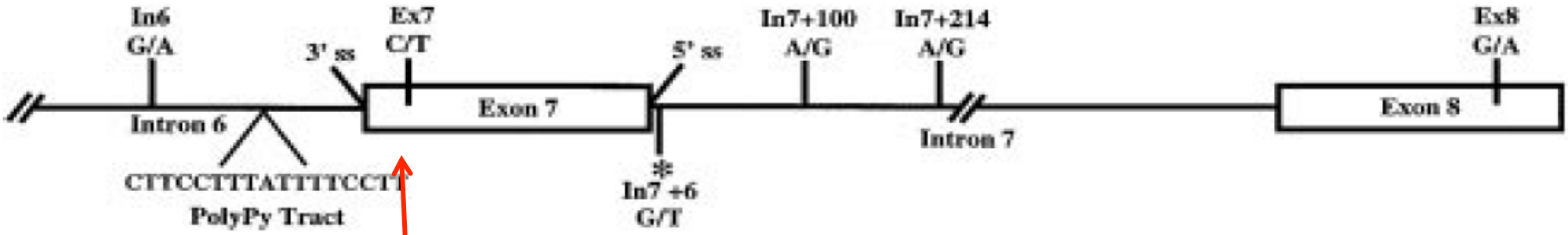
Mutations

- Up to **95%** of SMA patients (SMNI-III) have homozygous deletions for two exons (7&8) of both telomeric copies of the SMN gene (SMN^t)
- Up to **5%** of SMA patients have frameshift mutations, gene conversions and point mutation
- Exons 5 and 6 of $NAIP^t$ gene are deleted in approximately 50% of type I SMA and 18% of types II and III SMA
- $P44^t$ is lost or interrupted in 73% of SMA type I patients and 7% in types II and III

Genetics

- All SMA patients have reduced fl-smn protein :
  - Type 1 – 9%
  - Type 2 – 14%
  - Type 3 – 18%
  - Carriers – 45 -55%
- When levels approach 23% - motor neuron function is normal.

SMN2 ESE hampers exon inclusion



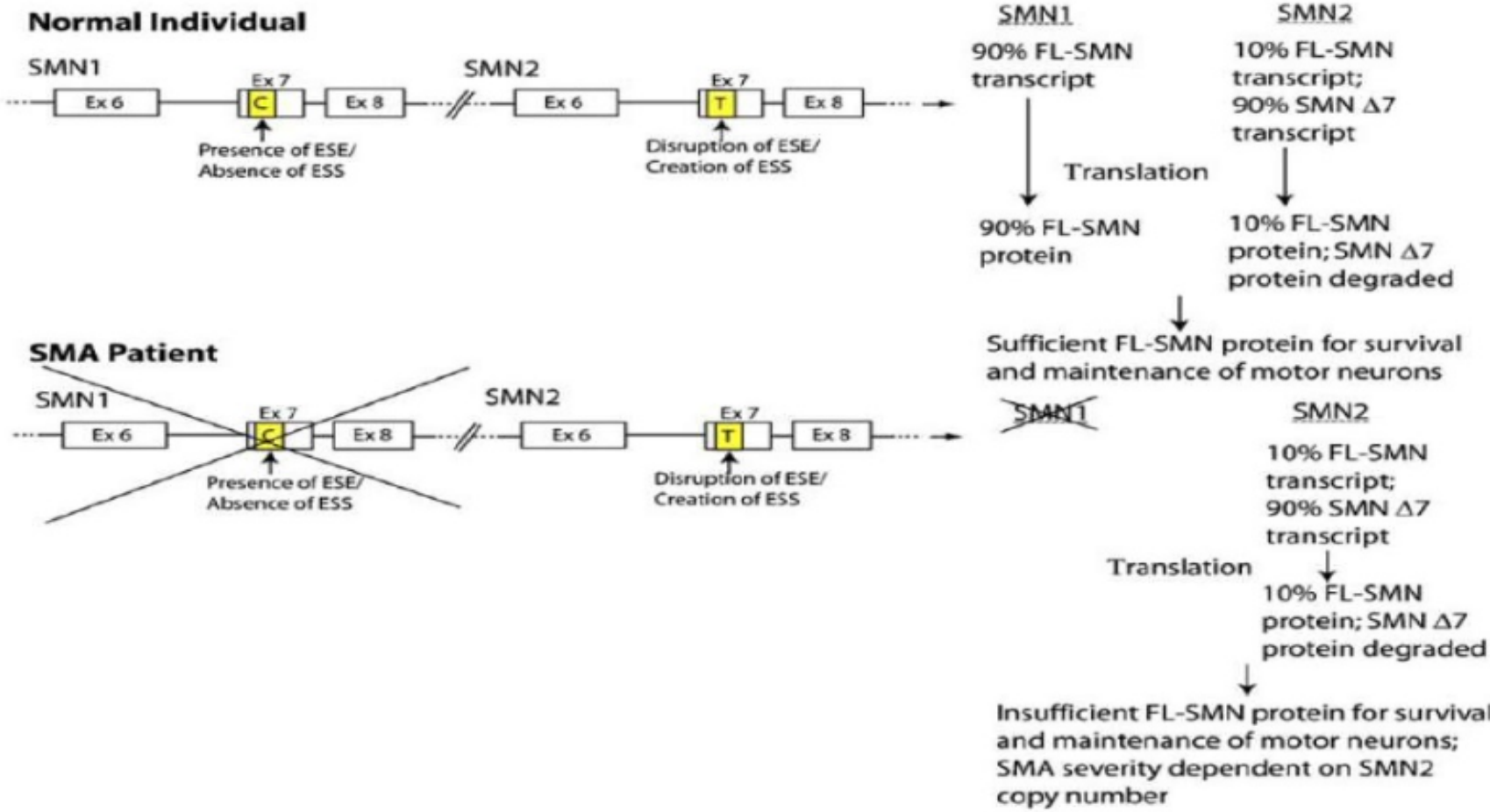
Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 6307–6311, May 1999
Genetics

A single nucleotide in the *SMN* gene regulates splicing and is responsible for spinal muscular atrophy

CHRISTIAN L. LORSON*†, ERIC HAHNEN†‡, ELLIOT J. ANDROPHY*§¶, AND BRUNHILDE WIRTH‡

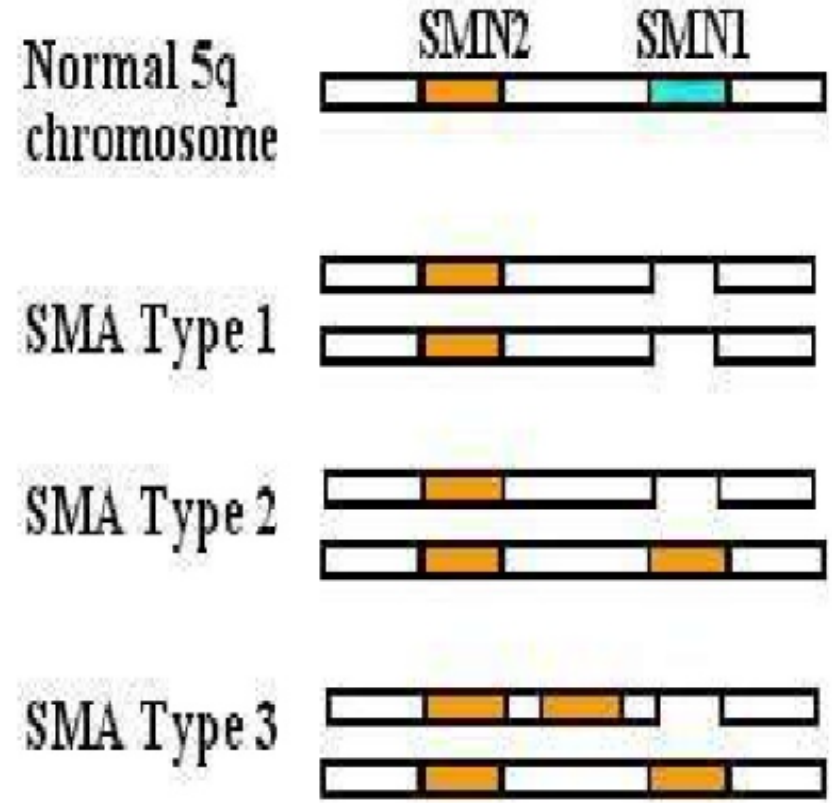
*Department of Dermatology, New England Medical Center, and §Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, MA 02111; and ‡Institute of Human Genetics, University of Bonn, D-53111 Bonn, Germany

Genetics


Normal Individual
SMN1
Ex 6
Ex 7
C
Ex 8
Presence of ESE/
Absence of ESS
SMN2
Ex 6
Ex 7
T
Ex 8
Disruption of ESE/
Creation of ESS
SMN1
90% FL-SMN transcript
Translation
90% FL-SMN protein
SMN2
10% FL-SMN transcript; 90% SMN Δ7 transcript
10% FL-SMN protein; SMN Δ7 protein degraded
Sufficient FL-SMN protein for survival and maintenance of motor neurons
SMA Patient
SMN1
Ex 6
Ex 7
C
Ex 8
Presence of ESE/
Absence of ESS
SMN2
Ex 6
Ex 7
T
Ex 8
Disruption of ESE/
Creation of ESS
SMN1
SMN2
10% FL-SMN transcript; 90% SMN Δ7 transcript
Translation
10% FL-SMN protein; SMN Δ7 protein degraded
Insufficient FL-SMN protein for survival and maintenance of motor neurons; SMA severity dependent on SMN2 copy number

Genetics

- SMA type I: Mutations
  - Mostly SMN1 deletions
  - Few missense point mutations in SMN1
  - SMN2 gene copy number: Often 2
- SMA type II
  - Mutations convert SMN1 gene to SMN2
  - SMN2 gene copy number: ≥ 3
  - Missense point mutations more common
- SMA type III
  - SMN2 gene copy number: ≥ 3
  - Missense point mutations more common



SMA DIAGNOSTICS

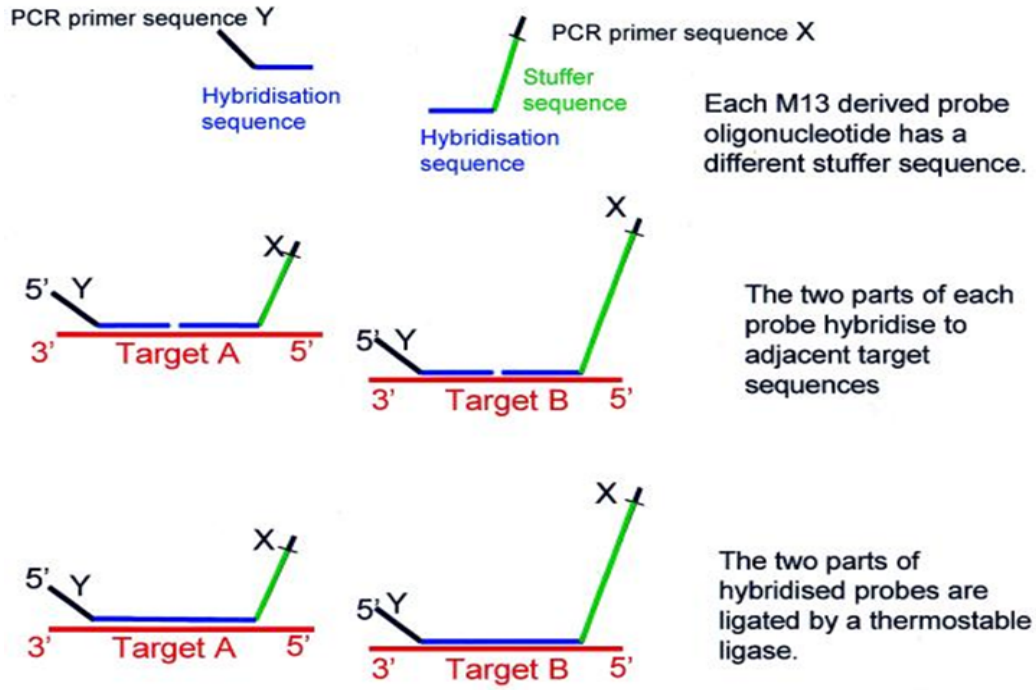
Reason for request/clinical indication (**COUNSELLING**)
positive family history for SMN1 gene deletion or SMA,
partner heterozygous for the deletion,
consanguinity with the partner,
SMA clinical diagnosis.

MOLECULAR TESTS

SMN1 copy number representation among tested subjects:
74% 2 copies
14% 1 copy
8% 3 copies
4% 0 copies

MLPA, Multiplex Ligation-dependent Probe Amplification

- Denatured genomic DNA is hybridized with a mixture of 40 probes.
- Each MLPA probe consists of two oligonucleotides, one synthetic and one M13-derived.



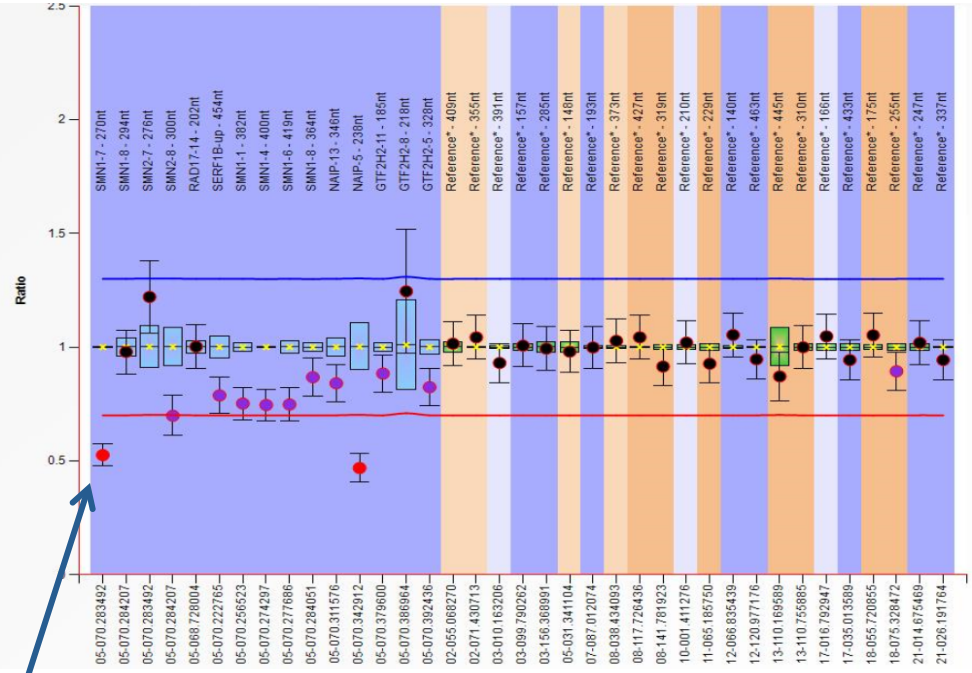
All probe ligation products are amplified by PCR using only one primer pair.

Amplification products are separated by electrophoresis. Relative amounts of probe amplification products reflect the relative copy number of target sequences.

- ***Diagnostic sensitivity of MLPA analysis***

- The SMA MLPA assay is a quantitative test for SMN1 gene copy number, and will not pick up subtle deletions, inversions or point mutations in SMN1 gene. Diagnostic sensitivity of the MLPA assay is additionally influenced by the fact that approximately 3-7% of the SMN1 alleles in the general population have two SMN1 copies on a single chromosome.

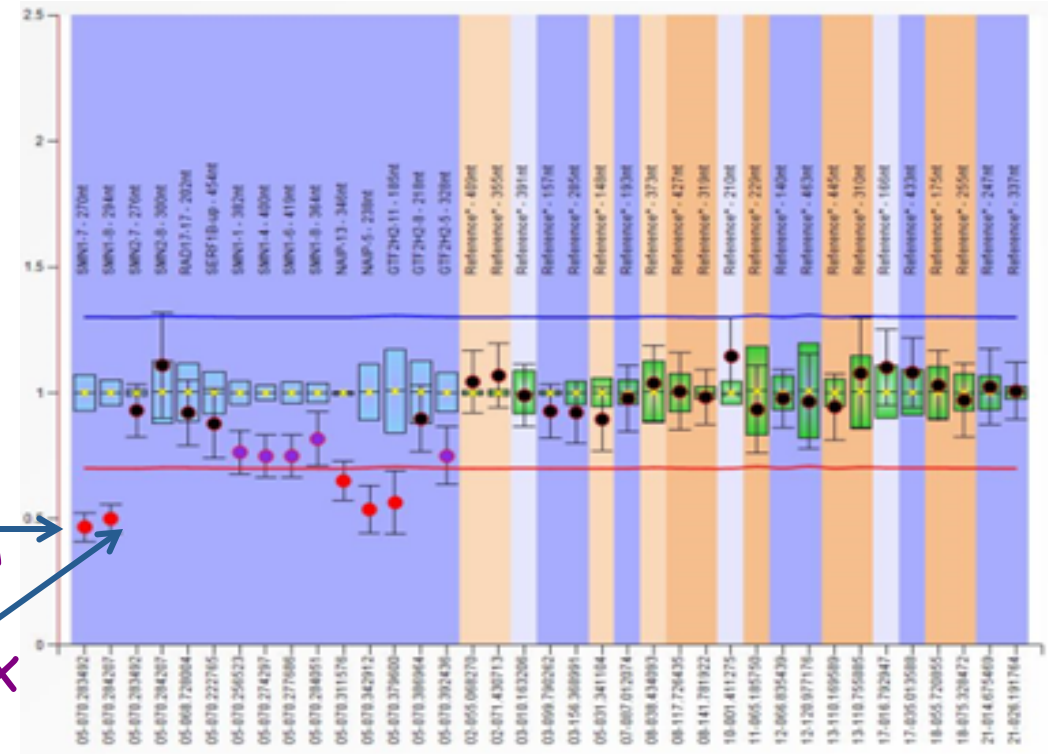
- Homozygous deletion of the SMN1 gene will be evident in approximately 95% of SMA Type I patients.

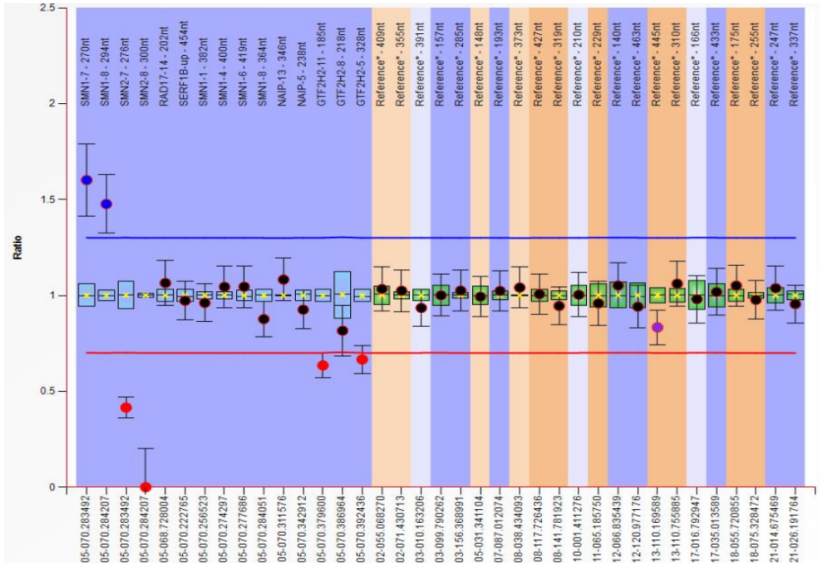


1 copy of SMN1 exon 7

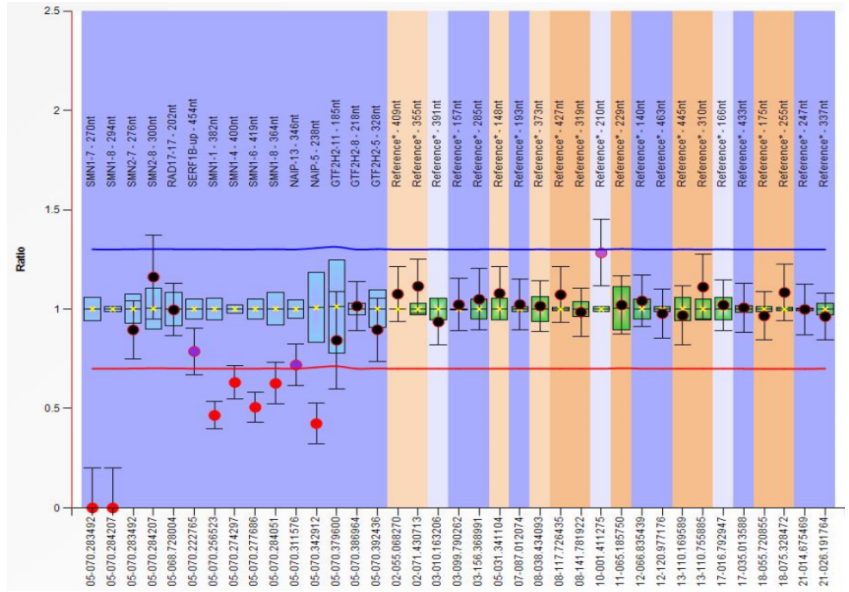
1 copy of SMN1 exon

1 copy of SMN1 ex

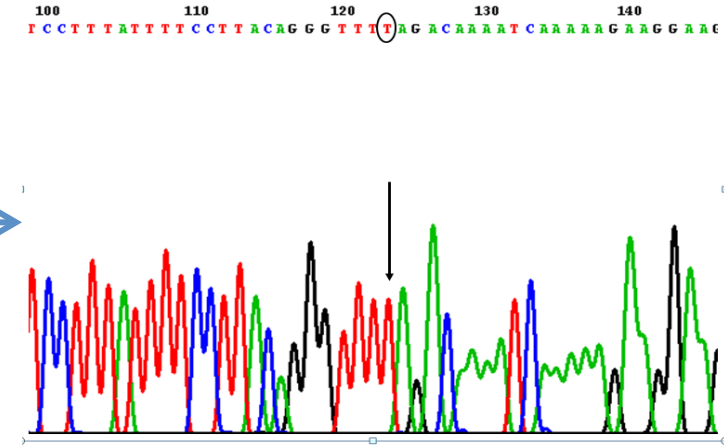




3 copies of SMN1 exons 7 and 8



0 copies of SMN1 exons 7 and 8



SMN genes sequencing

Table 1. Primer sequences and the length of the amplified *SMN* gene fragment

Gene *SMN*	Sequence	Annealing temperature, °C/*n*	
Exon 1	5'–3' CAGTGAAATGAAAGGATTGAGAGA 5'–3' CTGAGAGCGCTAATAGGGAGAC	62/30	
Exon 2A	5'–3' GTGTGGATTAAGATGACTCTTGG 5'–3' CAATTCCTTTCCAAATGAATAACGAG	62/30	
Exon 2B	5'–3' TGCACCACCCTGTAACATGTAC 5'–3' CACTATTAAATAAGGACTAATGAGACATCC	62/30	
Exons 3–4	5'–3' CAAATCTCTACCCTCTATCCTTCAC 5'–3' CAAATTAGTTAACAGAGAGGTTAAATGTCC	62/30	
Exon 5	5'–3' GGTTTTGAGTCCTTTTTATTCCTATC 5'–3' GTGAAAGCAAAATCTAACCTATACTC	62/30	
Exon 6	5'–3' GCATTCACTATATAATATAAATCTCCAGAC 5'–3' CAAAAAAATTGTCAGGAAAAGATGC	62/30	231
Exon 7	5'–3' GTGAAACAAAATGCTTTTTAACATCC 5'–3' CCATAAAGTTTTACAAAGTAAGATTCAC	62/30	206
Exon 8	5'–3' GGTTTAACTGGAATTCGTCAAGC 5'–3' CAAATTTTCTCAACTGCCTCACC	62/30	383

Table 2. Localization and the type of point mutations in type I–III SMA patients

	DNA no.	SMA type	Genotype	Mutation		Exon, intron	Origin
				cDNA	protein		
1	7830	I	1T/2C	c.43C>T	p.Gln15X	E1	*de novo*
2	8251	III	1T/3C	c.684dupA		E5	From father
3	7313	I	1T/2C	c.815A>G	p.Tyr272Cys	E6	"
4	7864	III	1T/3C	c.821C>T	p.Thr274Ile	E6	"
5	7111	I	1T/2C	c.824G>C	p.Gly275Ala	E6	"
6	7413	I	1T/3C	c.824G>C	p.Gly275Ala	E6	"
7	7643	I	1T/2C	c.835-2A>T		I6	"
8	8022	I	1T/2C	c.836G>T	p.Gly279Val	E7	From mother

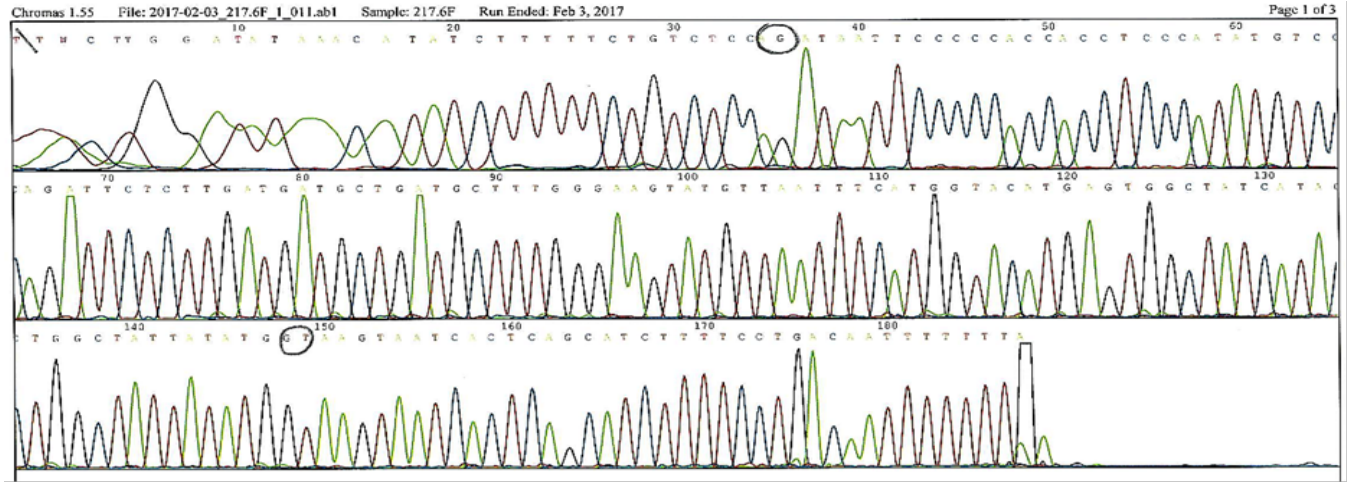
T, telomeric gene copy *SMN* (*SMN1*); C, centromeric gene copy *SMN* (*SMN2*).

Table 3 Guidelines for the clinical indications for DNA analysis

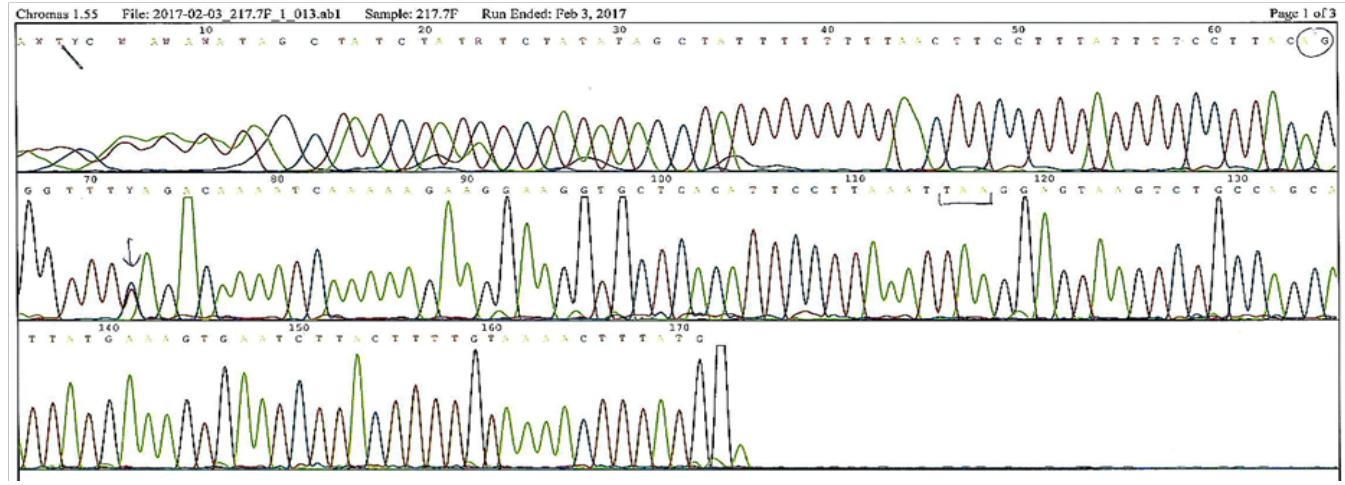
Clinical presentation	*Test*
Any reasonable suspicion of SMA	Homozygous SMN1 exon 7 deletion test
Clinical criteria supporting the diagnosis of SMA types I, II, or III should be in agreement with those described,[40] including characteristic abnormalities in a muscle biopsy	Hemizygous SMN1 exon 7 deletion test/SMN1 subtle mutation screening

Zabnenkova et al. 20

SMN genes sequencing



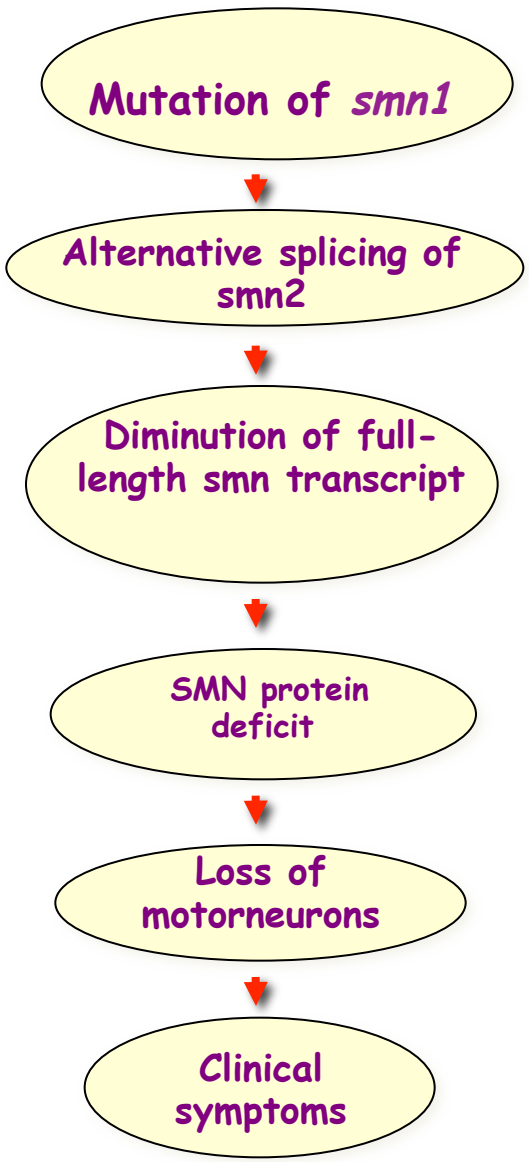
SMN exon 6



SMN exon 7

Clinical trials and outcome measures in SMA

Therapeutic targets for SMA



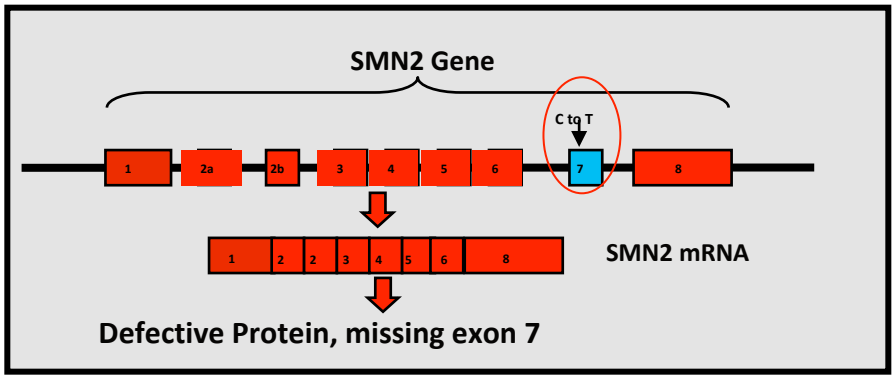
	example
Replacement of SMN1	Gene therapy
Inclusion of exon 7	**Antisense** Pharmacological upregulation
Increase of SMN transcripts	HDAC inhibitors Hydroxyurea Quinazolones Aminoglycosides
Stabilisation of SMN protein	Indoprofen Proteasome inhibitors Polyphenols
Neuroprotection	Riluzole, **TRO19622** Neurotrophic factors
Cell therapy	Stem cells

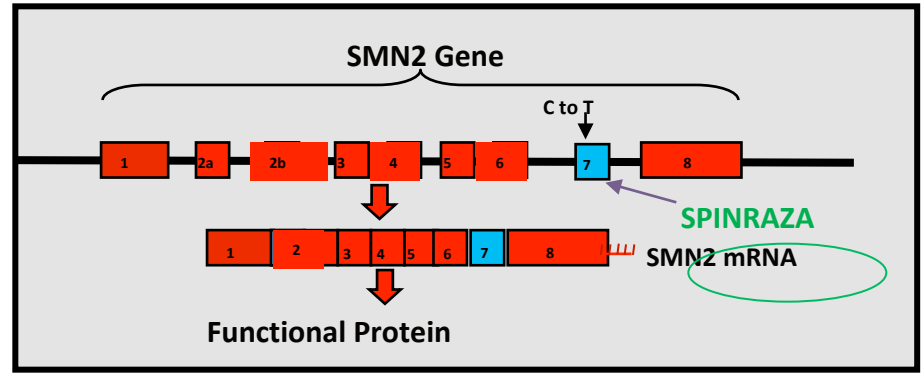
Courtesy of Francesco Muntoni)

SPINRAZA: Modulating Splicing of SMN2 to Increase Normal SMN Protein

Modifying splicing

- Chimeric 2'-*O*-methoxyethyl modified (MOE) antisense drug
- **Corrects the splicing disorder in SMN2**, resulting in the **production of fully functional SMN protein** in model systems
- In mild and severe mouse models of SMA provides a phenotypic and pathological benefit when delivered centrally*
- Distributes broadly to spinal cord motor neurons after intrathecal delivery in monkeys*
- Has a long half life in CNS tissue (>6 months in animal models)





*(Hua et al., Genes Dev., 2010; Passini et al., Sci Transl Med, 2011; Hua et al., Nature, 2011)

THE Spinraza trial
come un farmaco posa cambiare la storia clinica di una malattia

- https://www.youtube.com/watch?v=3EKs8GJnkrc

Clinical Program SPINRAZA

- Orphan Drug Status in US and EU; Fast Track Designation in USA and Europe
- Phase 1 Single-dose Study in Children with SMA – completed

- Phase 1b/2a Multiple-dose Study in Children with SMA - completed

- Phase 2 Multiple-dose Study in Infants with SMA - completed

  - SPINRAZA AVAILABLE FOR SMA TYEP 1-2-3
  - SMN2 copy number-dependent

Clinicaltrials.gov

- https://clinicaltrials.gov/