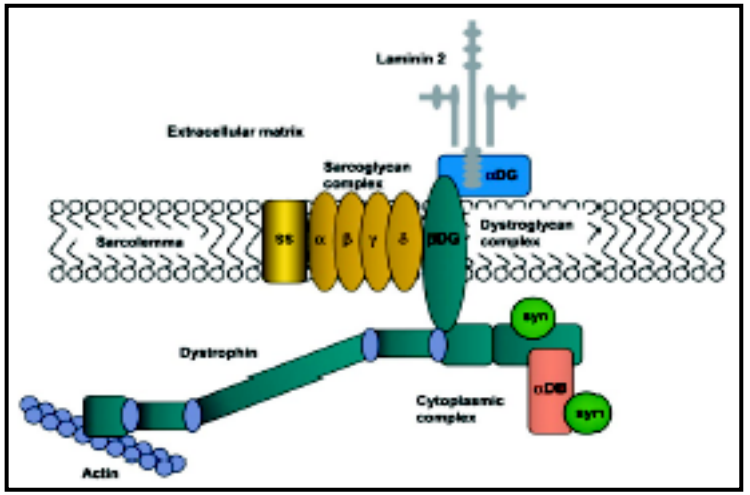
The exon skipping strategy in the therapeutic treatment of Duchenne Muscular Dystrophy

Duchenne Muscular Dystrophy (DMD)

- X-linked recessive disorder
- affects 1 in 3500 live males
- DMD muscles degenerate with activity
- leads to ***death*** by the third decade of life



The gene is too big for a classical gene therapy intervention

Dystrophin

- protein= 427 KDa
- DNA= 2,5 Mb
- cDNA= 14 Kb

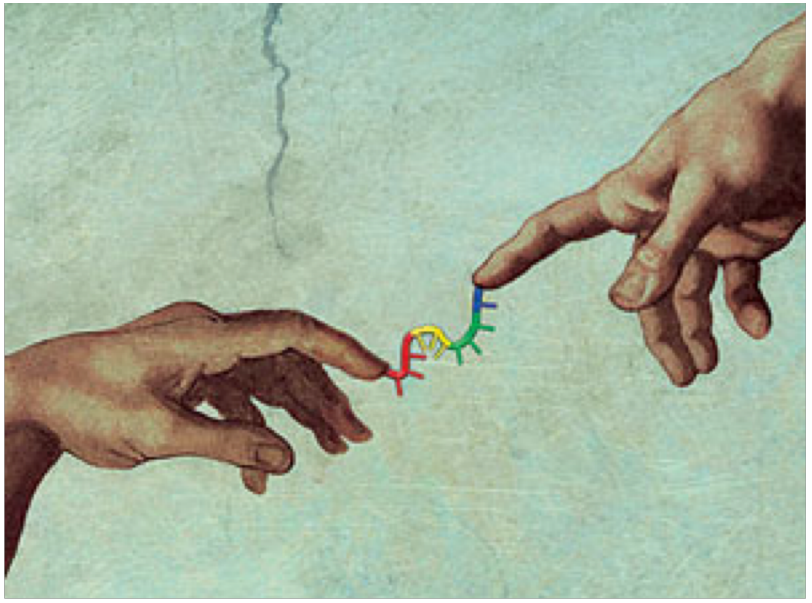
A new strategy: **EXON SKIPPING**

Modify the mutated dystrophin mRNA through the use of **antisense RNA molecules**

- RNA molecules can interfere with gene expression in a sequence-specific way

- The specificity is extremely high and can be obtained with molecules of low complexity

- Non-immunogenic



Economist.com

The RNA revolution

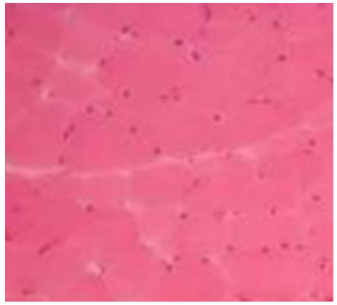
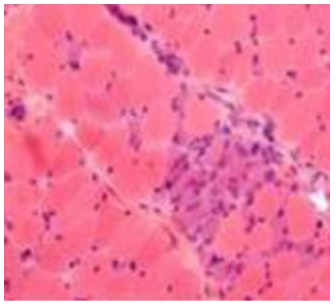
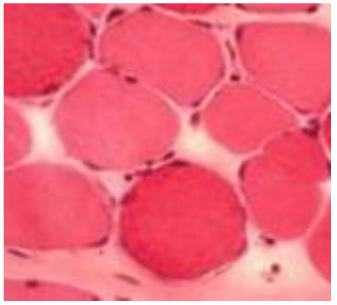
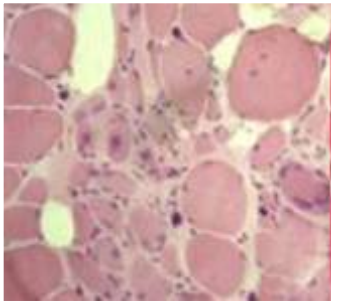
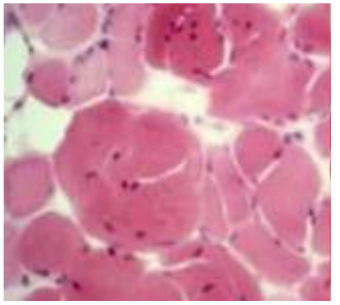
Biology's Big Bang

Duchenne Muscular Dystrophy (DMD)



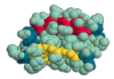
is a severe disorder characterized by rapid progression of muscle degeneration leading to loss of ambulation and death.

Histopathology of a Duchenne muscle

La Distrofia Muscolare di Duchenne

30 ANNI DI RICERCA



1868
Prima descrizione della Duchenne
Guillaume Duchenne De Boulogne

1987
Identificata la proteina associata al gene

1994
PPMD

Duchenne Parent Project

1996
PPO

2008
Primo trial per **Exon skipping 51**

2011
Trial italiano con **terapia cellulare**

2016
Primo farmaco approvato in **US Exondys51**

OGGI
Oltre 30 tria nel mondo 10 in Italia

1986
Scoperto **il gene della distrofina**

Anni '90
inizia la ricerca sulla DMD

2000
Studi preclinici su terapie innovative

2004
Primo trial per **mutazioni nonsenso**

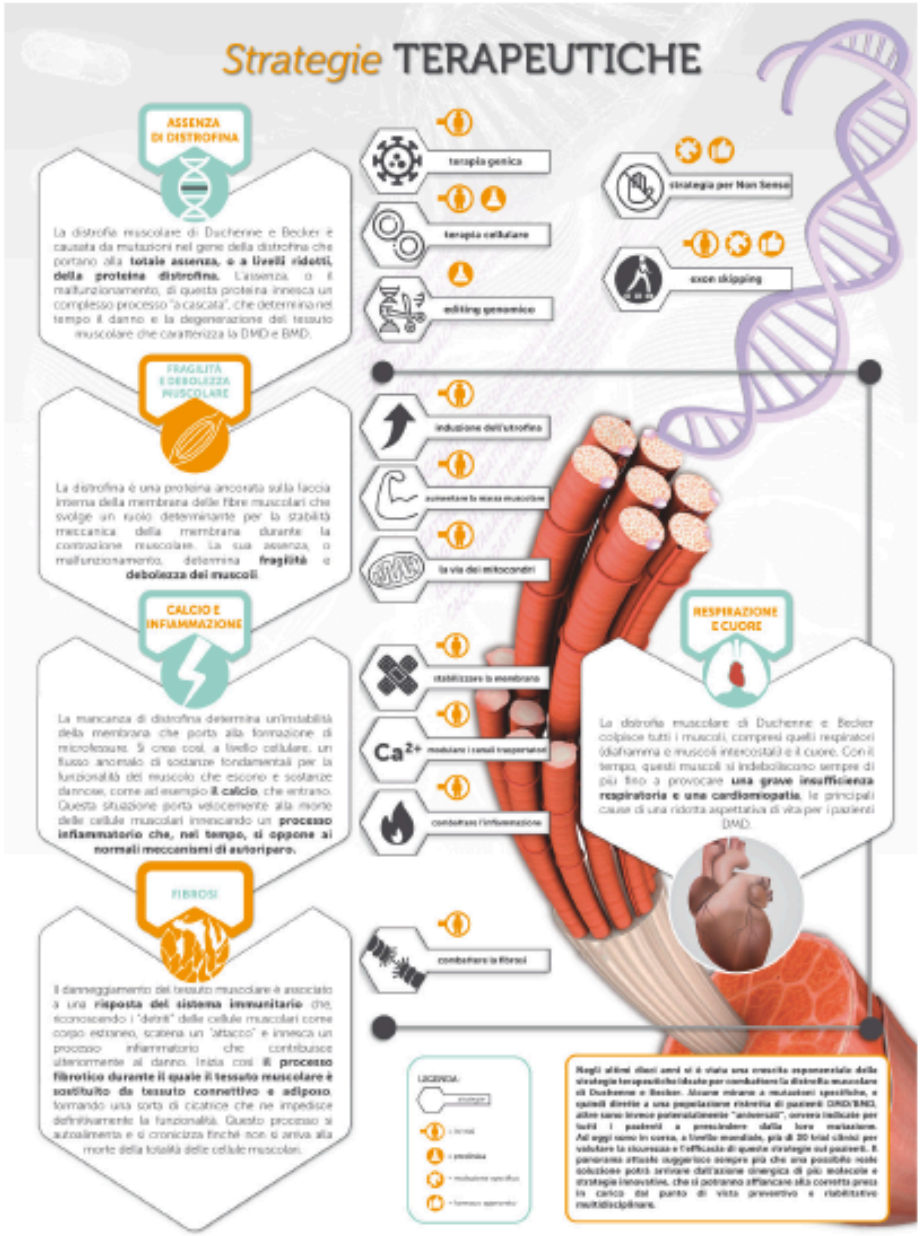
2010
Prime Linee Guida DMD

2014
Primo farmaco approvato in **EU Translarna**

Primi studi preclinici con **CRISPR**

2017-18
Avviati 3 trial con **terapia genica**

La Distrofia Muscolare di Duchenne



La Distrofia Muscolare di Duchenne

Terapia approvata

Esperimenti in laboratorio

Studi clinici nell'uomo

Valutazione post-approvazione

Preclinica	Fase 1	Fase 2	Fase 3	Fase 4
Prova di concetto	Farmacologia umana	Esplorazione terapeutica	Conferma terapeutica	Farmacovigilanza Valutazione a lungo termine

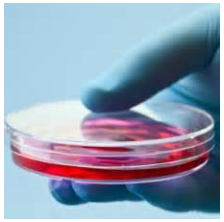
3-5 anni

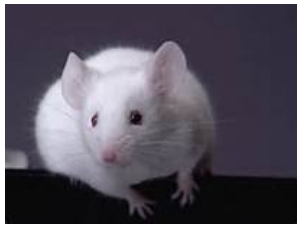
6-10 anni

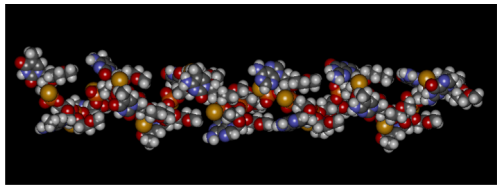
2-5 anni

10.000 molecole

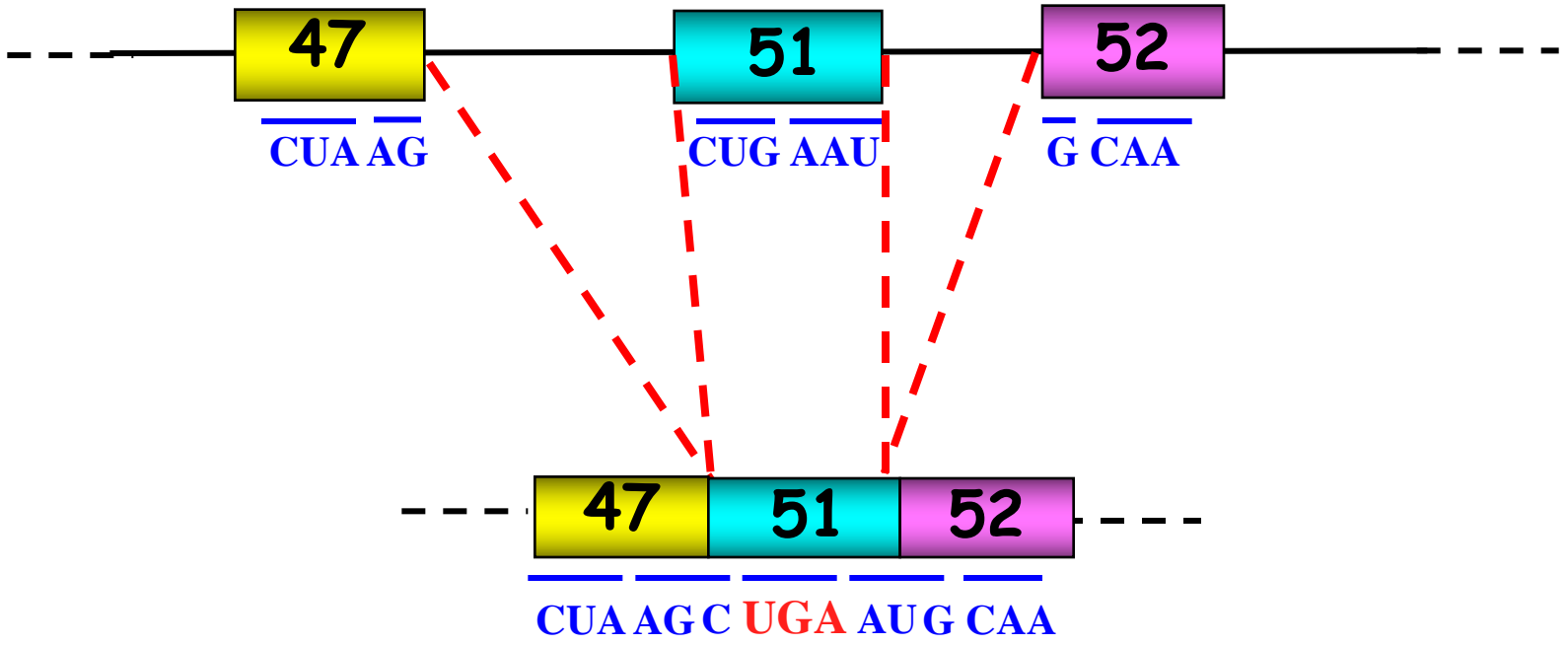
1 molecola





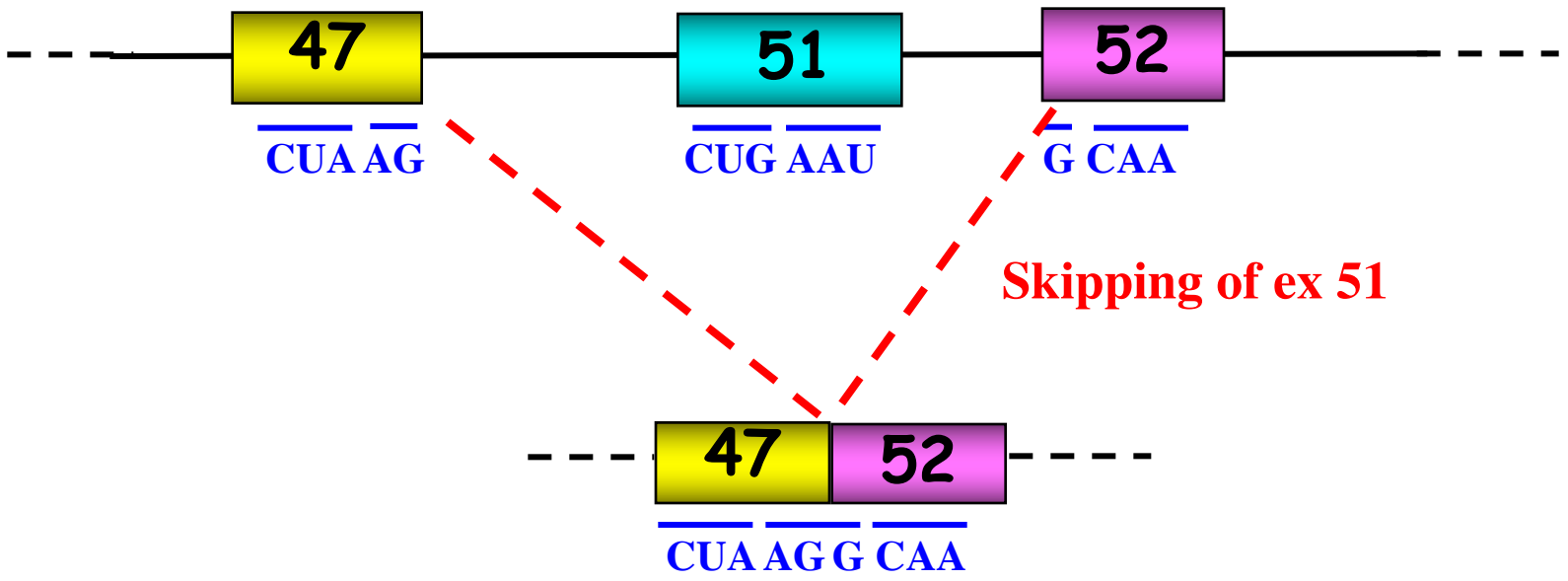


Duchenne Muscular Dystrophy
- the 48-50 deletion -



out-of-frame fusion → **UGA** (stop codon) → **premature translation termination**

Exon skipping can revert the phenotype



In-frame mRNA → **translation of a shorter but still functional protein**

- Becker-type -

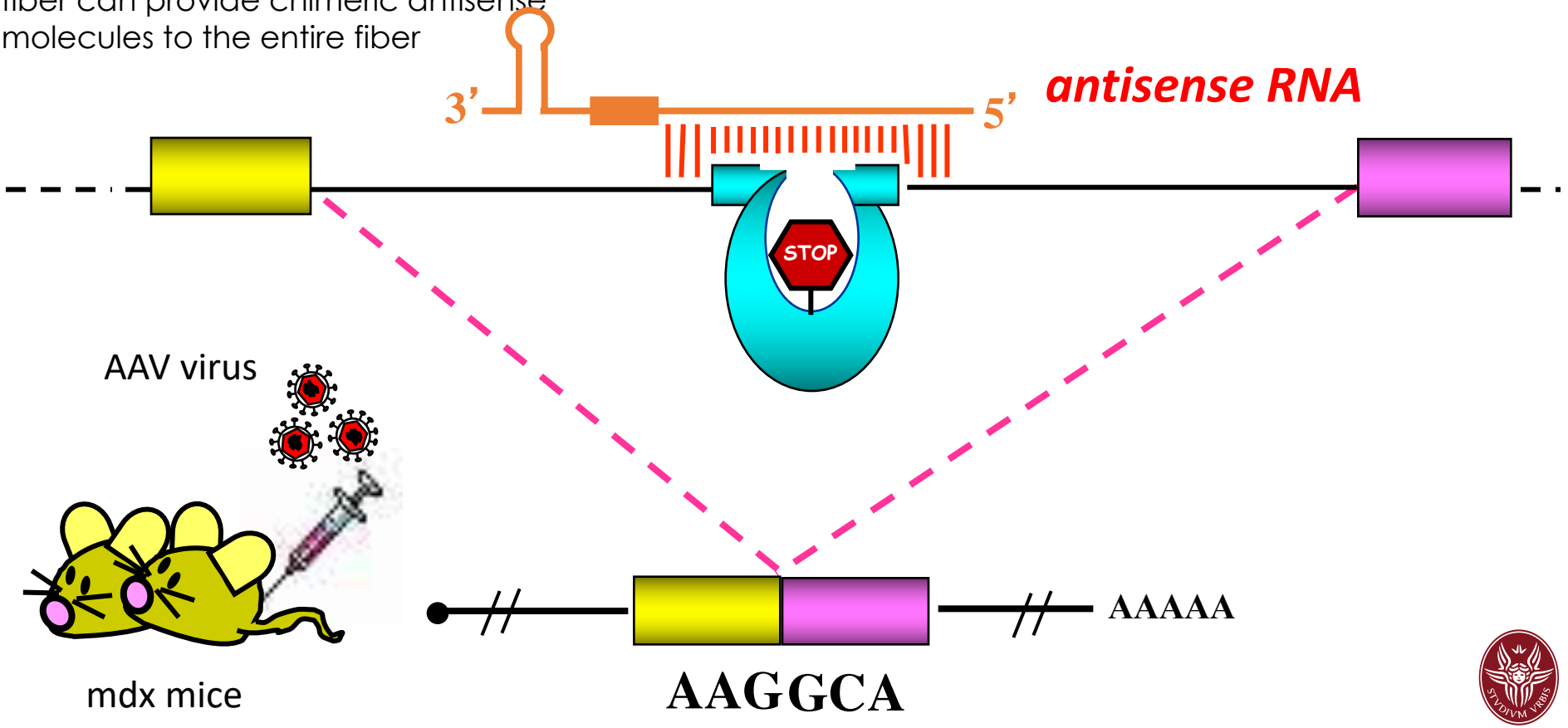
75% of all known dystrophin mutations can be cured by exon skipping

skipping of ex 51 - 18%

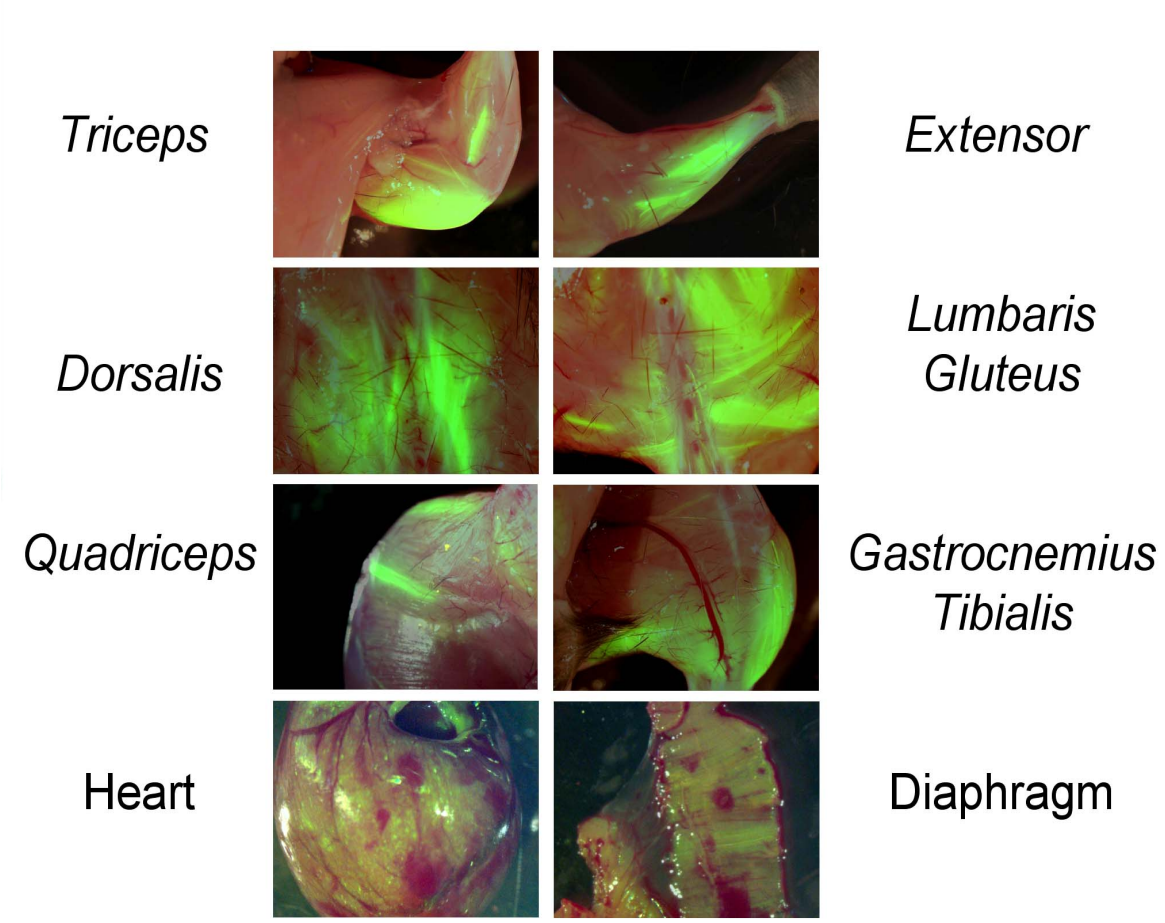
Antisense RNA technology applied to the correction of DMD mutations

U1 snRNA

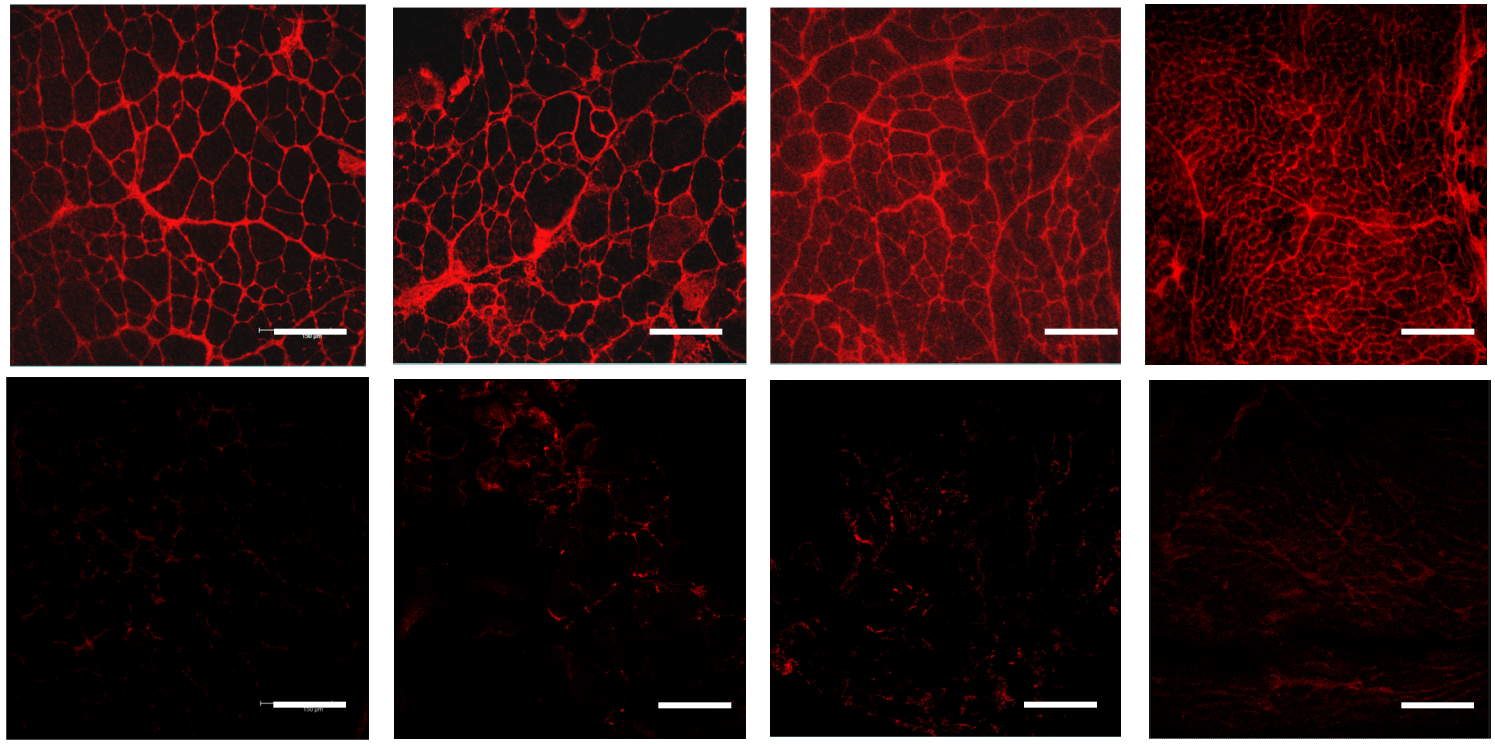
- nuclear RNA with specific recognition for splice junctions
- is matured in the cytoplasm an then reimported in the nucleus
- few transduced nuclei in the muscle fiber can provide chimeric antisense molecules to the entire fiber



AAV-mediated gene transfer allows a genome wide transduction of all muscle districts



The therapeutic benefit lasts for the entire life of the animal



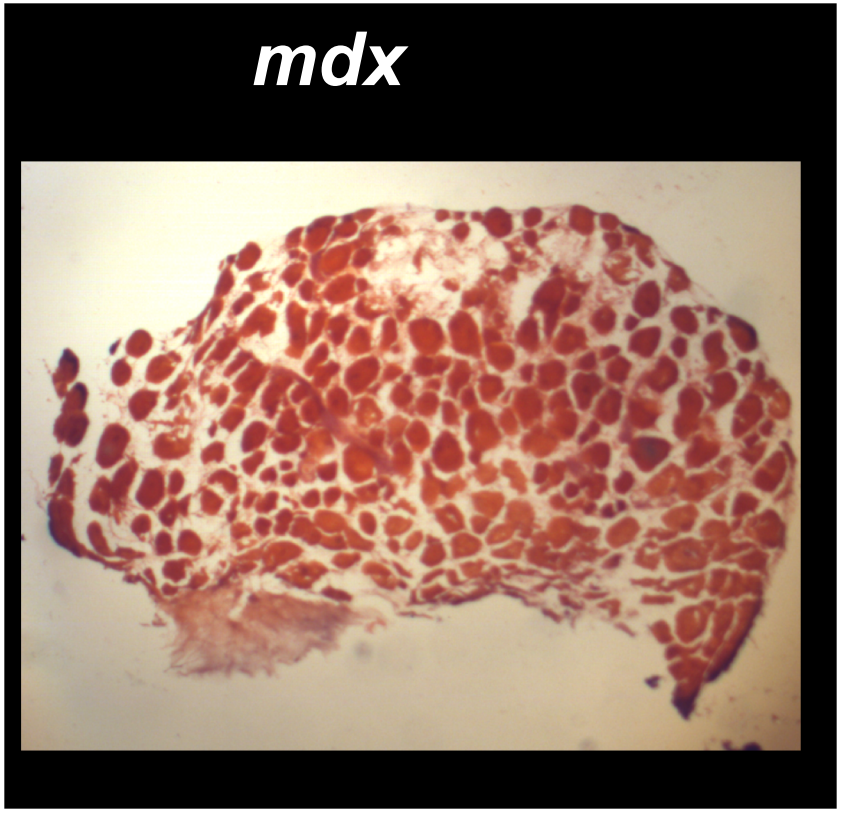
Mice are injected at 6 weeks and sacrified at 20 months

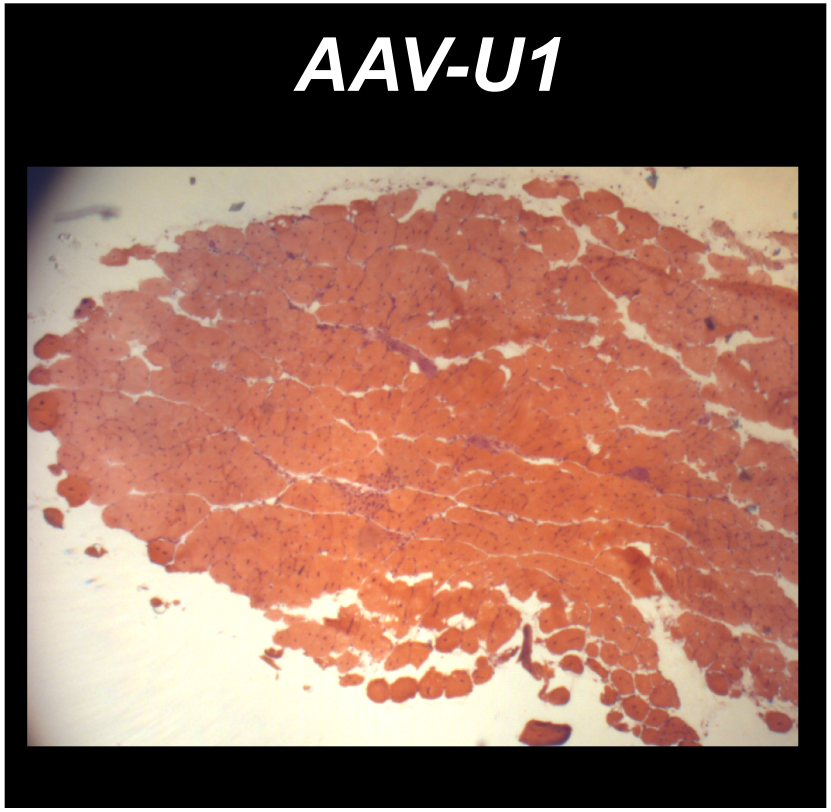
Dystrophin expression is maintained for such long time

Denti *et al.*, Hum Gen Ther

Long-term benefit of exon skipping treatment

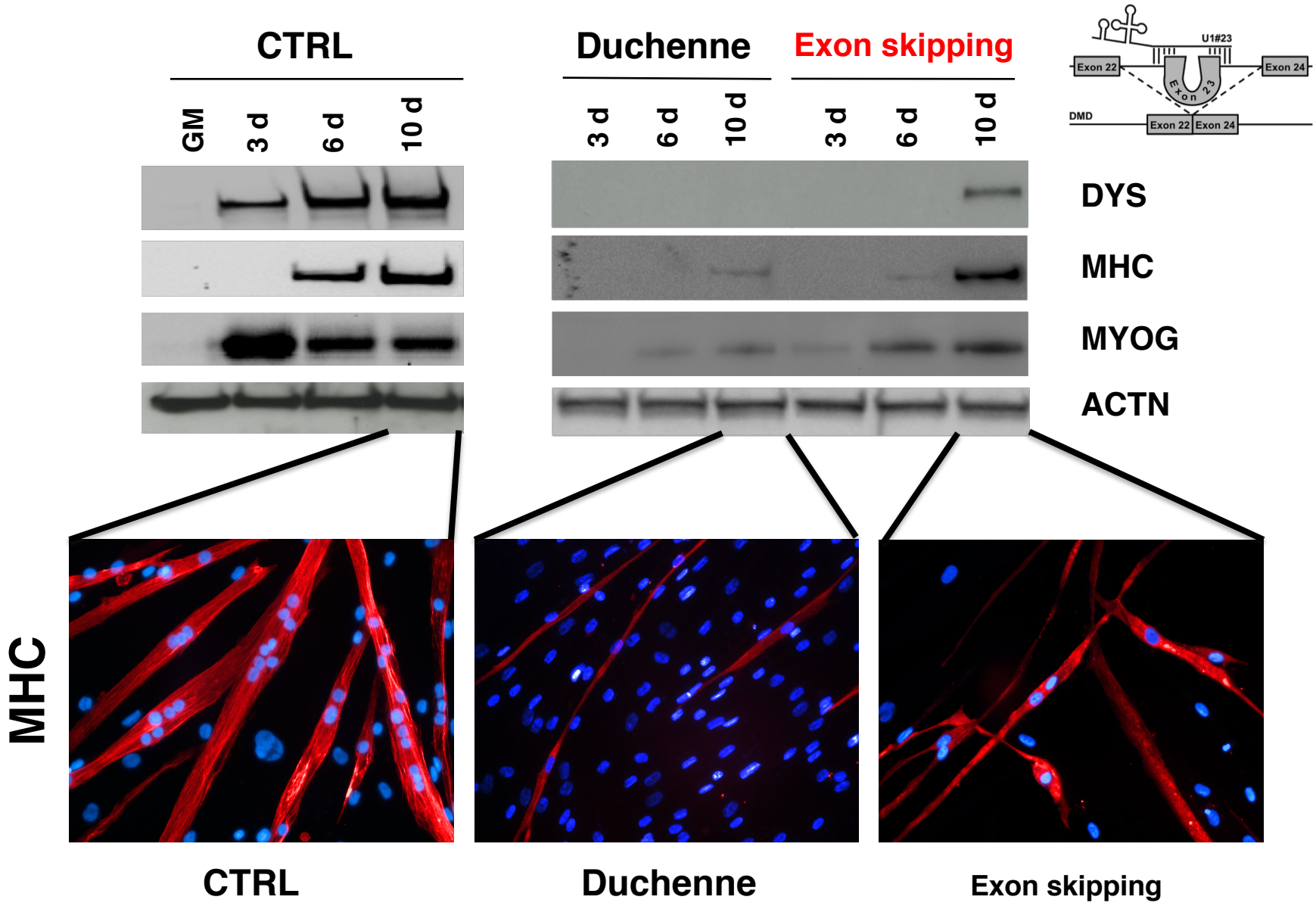
18 months after AAV injection





The morpho-functional benefit obtained in exon skipping-treated animals was much stronger than what expected for the amount of rescued dystrophin in U1#23 (1-10%)

Exon skipping rescues dystrophin expression and correct timing of myogenic marker expression



Therapeutic approaches to DMD

	OBIETTIVO	STRATEGIA	APPROCCIO
	Ripristinare la produzione di distrofina	Fornire il gene sano in grado di produrre la distrofina	Terapia genica
		"Riparare" la mutazione genetica in maniera tale da avere un ripristino della distrofina	Exon skipping
			Mutazioni non senso
	Rinforzare il muscolo /Ridurre la fragilità muscolare	Stimolare la formazione di complessi alternativi alla distrofina	
		Aumentare la massa muscolare	
		Migliorare il metabolismo muscolare	
	Contrastare la degenerazione muscolare	Limitare l'accumulo di calcio nelle cellule muscolari	
	Ridurre l'infiammazione	Ostacolare l'infiammazione cronica agendo sui principali protagonisti del processo	
	Ridurre la fibrosi	Ostacolare la fibrosi agendo sui principali protagonisti del processo	
	Contrastare il deficit cardiaco	Ostacolare la fibrosi cardiaca agendo sui principali protagonisti del processo	

Oligonucleotides in DMD therapy

EXONDYS 51 (ETEPLIRSEN) - Fase 3

Italia, Regno Unito, Francia, Germania, Belgio, Stati Uniti

Eteplirsen, anche noto con il nome commerciale EXONDYS 51, è un oligonucleotide antisenso (AON) di tipo morfolino fosforodiamidato (PMO), sviluppato da Sarepta Therapeutics per il trattamento dei pazienti DMD con una delezione nel gene della distrofina potenzialmente trattabile con lo skipping dell'esone 51. Tali pazienti rappresentano circa il 13% della popolazione Duchenne.

Casimersen - Fase 1/2

Stati Uniti

Casimersen, precedentemente noto come SRP-4045 è un oligonucleotide antisenso (AON) che impiega un morfolino fosforodiamidato (PMO). La molecola è sviluppata dall'azienda Sarepta Therapeutics per indurre lo skipping dell'esone 45 del gene della distrofina.

ESSENCE - Fase 3

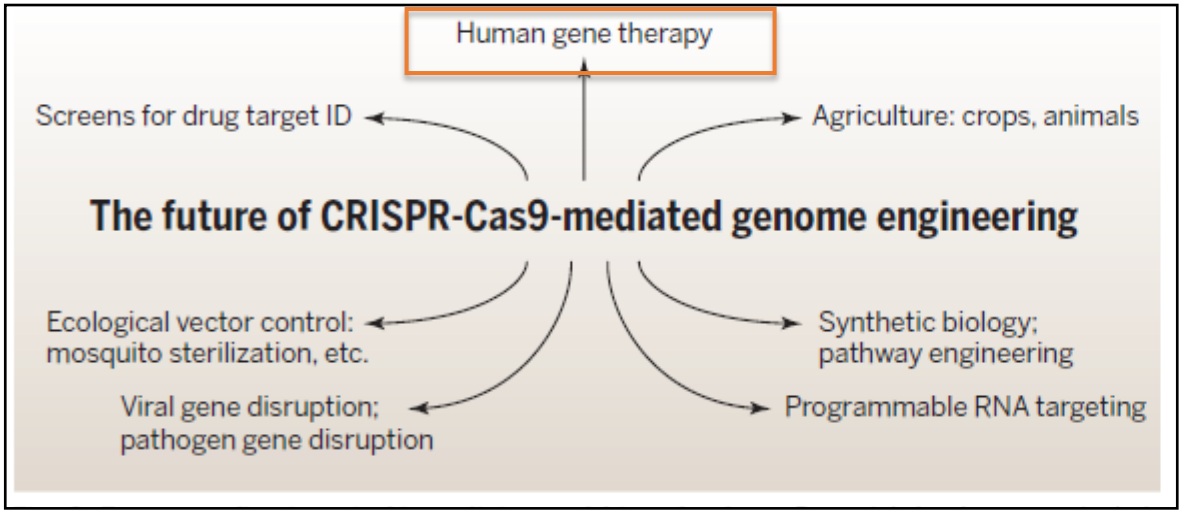
Italia, Regno Unito, Francia, Germania, Spagna, Svezia, Belgio, Repubblica Ceca, Israele, Stati Uniti

Questo studio clinico coinvolge casimersen e golodirsen, precedentemente noti come SRP-4045 e SRP-4053, due oligonucleotidi antisenso (AON) che impiegano un morfolino fosforodiamidato (PMO). Entrambe le molecole sono sviluppate da Sarepta Therapeutics per indurre lo skipping rispettivamente dell'esone 45 e dell'esone 53 del gene della distrofina.

Genome editing

CRISPR/Cas9 - un'innovativa tecnica di ingegneria genetica che permette di effettuare **correzioni direttamente sul DNA in maniera specifica e definitiva**

Genome editing



Doudna and Charpentier, Science 2014

Application of CRISPR/Cas9 genome editing to the study and treatment of disease.

Application of genome editing technologies to the study and treatment of hematological disease

[Application progress of CRISPR/Cas9 genome editing technology in the treatment of HIV-1 infection].

[Article in Chinese]

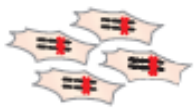
Application Progress of CRISPR/Cas9 System for Gene Editing in Tumor Research

Chao LIU, Zhiwei LI, Yanqiao ZHANG

In vivo gene therapy potentials of CRISPR-Cas9

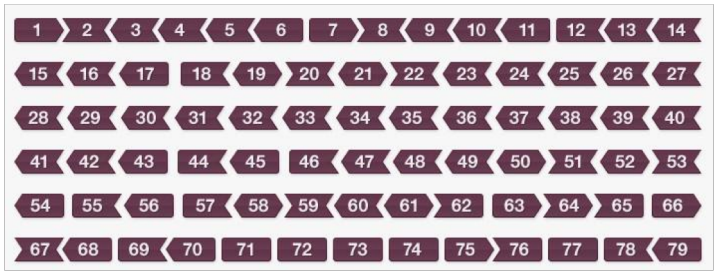
H-Y Xue[1,6], X Zhang[2,6], Y Wang[3,6], L Xiaojie[3], W-J Dai[4] and Y Xu[5]

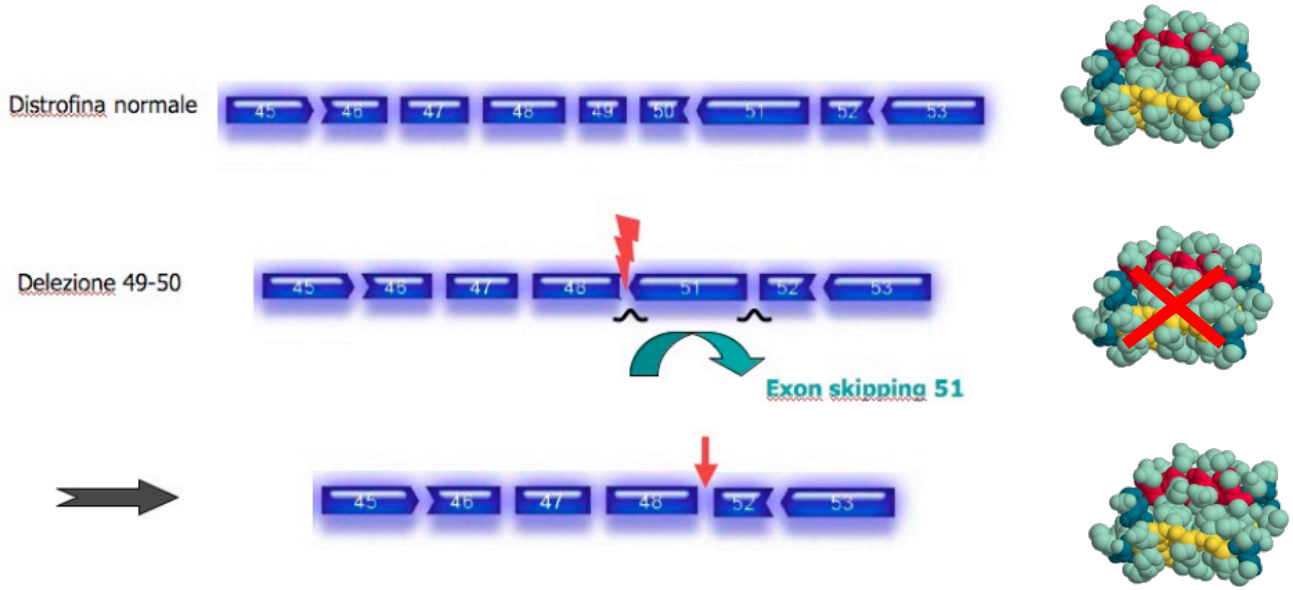
Genome editing

 *In vivo* animal studies	 Cell-based studies
Crygc-associated cataract: 1bp deletion in exon3	HIV-1 resistance: editing CCR5
Fah mutation-related tyrosinemia in hepatocytes: point mutation in exon 8	β-thalassemia: correction of human hemoglobin β-associated β-thalassamia mutations
Reduction cholesterol levels: *PCSH9* knockout mice	Cystic fibrisis transmembrane conductor receptor (CFTR): CFTR exon 11
Duchenne's muscular dystrophy (DMD): *dmd* dystrophin gene correction	Duchenne's muscular dystrophy (DMD): dmd dystrophin gene correction

Pellagatti et al.,Arch Toxicol 2015

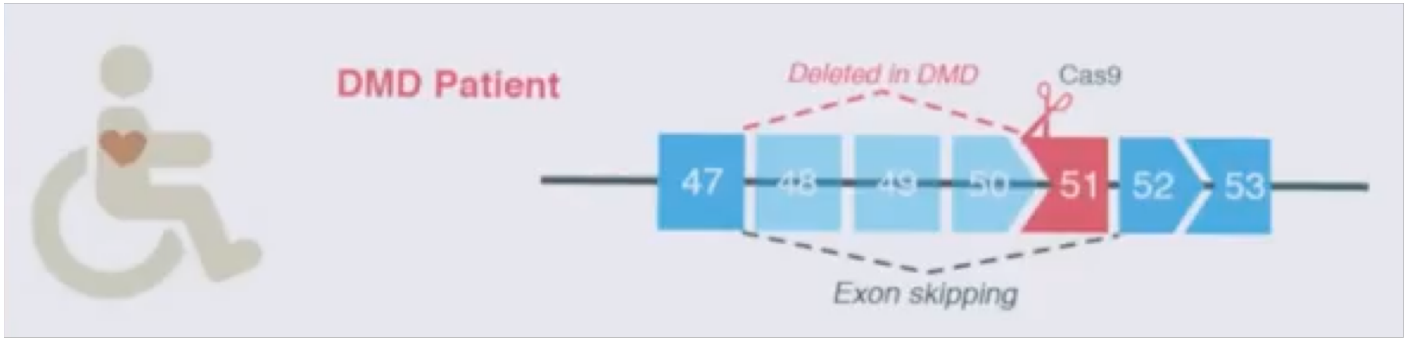
Exon skipping





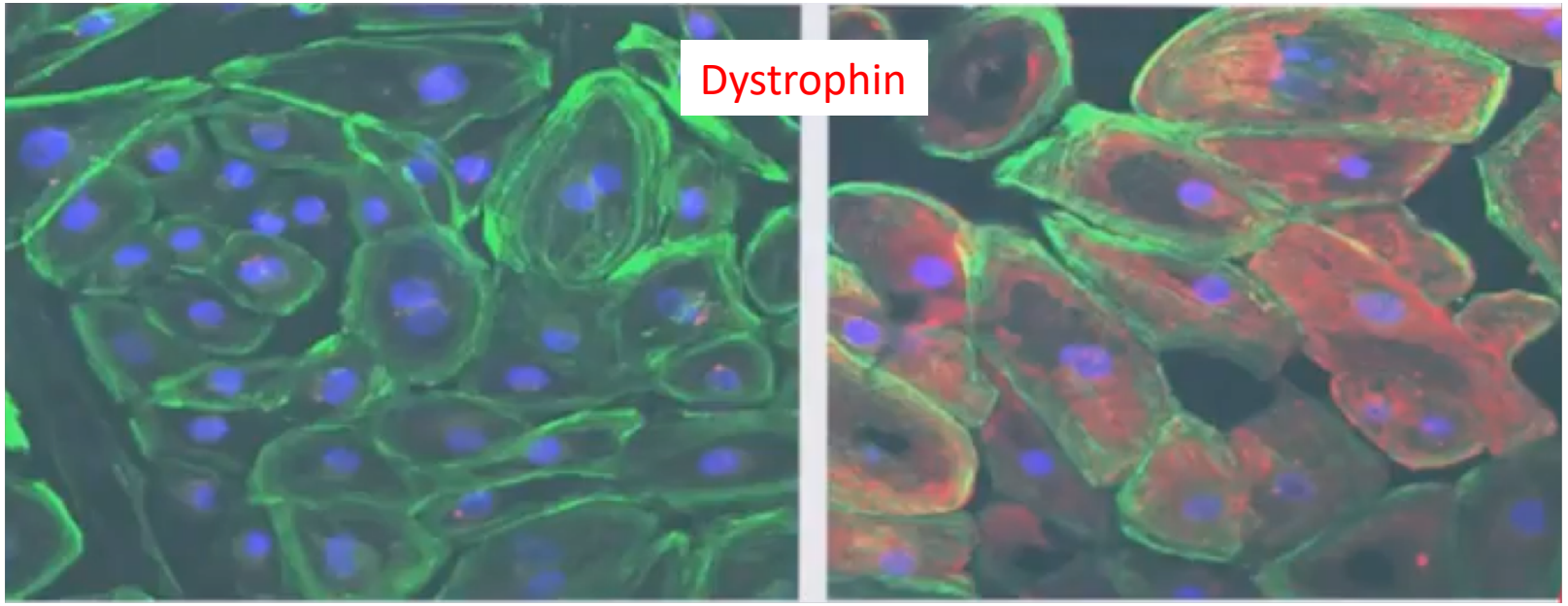
Ristabilire lo **schema di lettura del gene** della distrofina che è stato modificato dalla mutazione
La distrofina prodotta sarà più corta del normale ma funzionale

Editing of human dystrophic cells



DMD cardiomyocytes

Myoedited
DMD cardiomyocytes

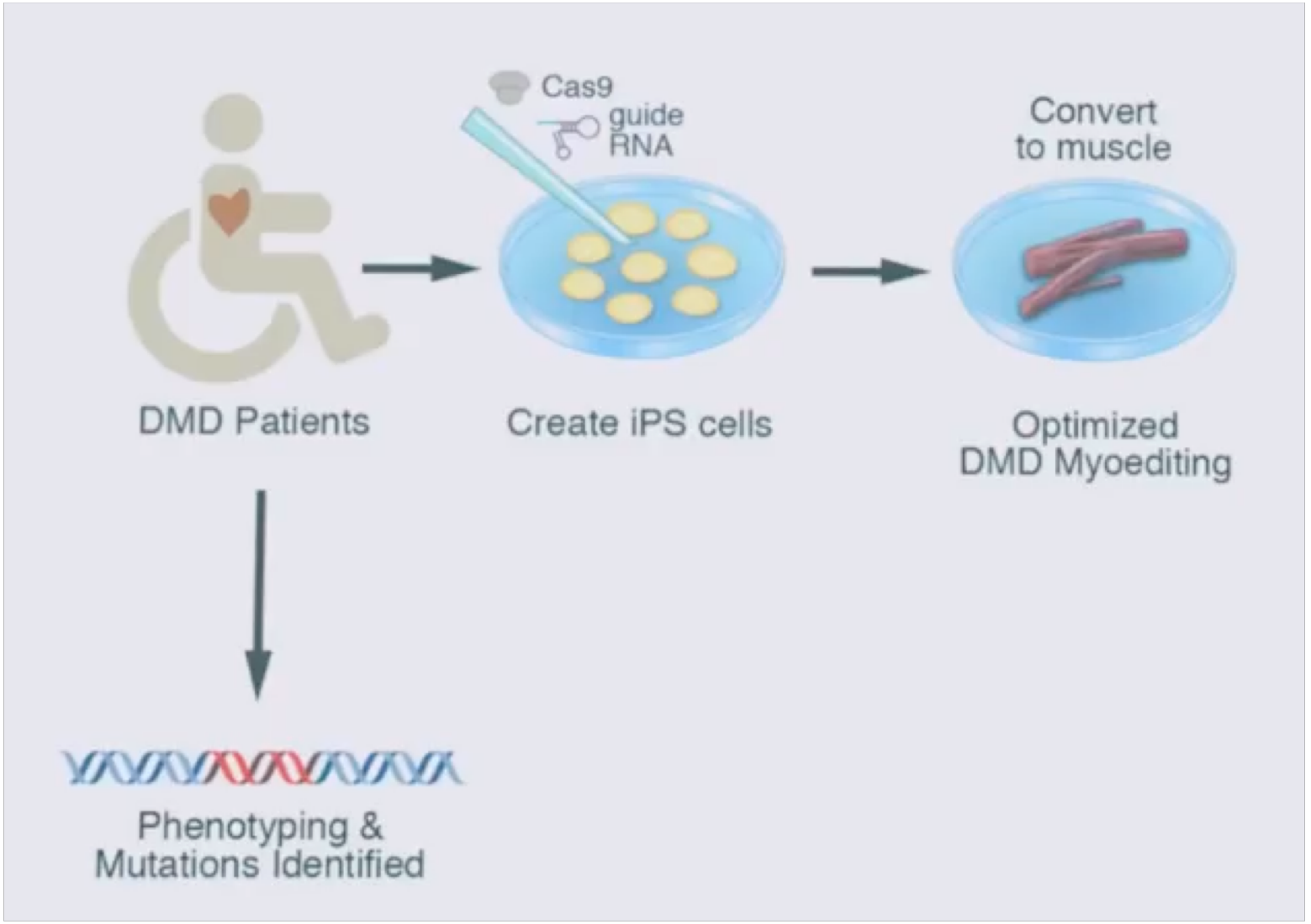


Dystrophin negative

Dystrophin positive

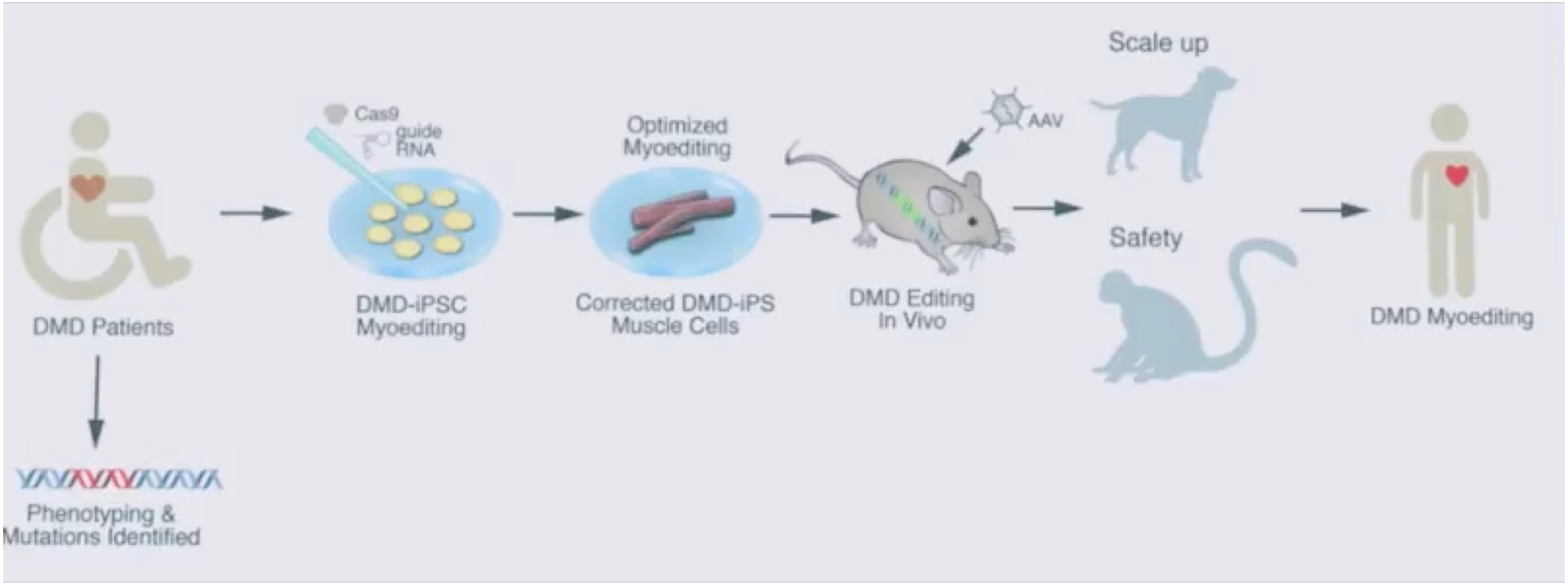
Zhang et al., 2017

Editing of human dystrophic cells



Zhang et al., 2017

Editing of human dystrophic cells



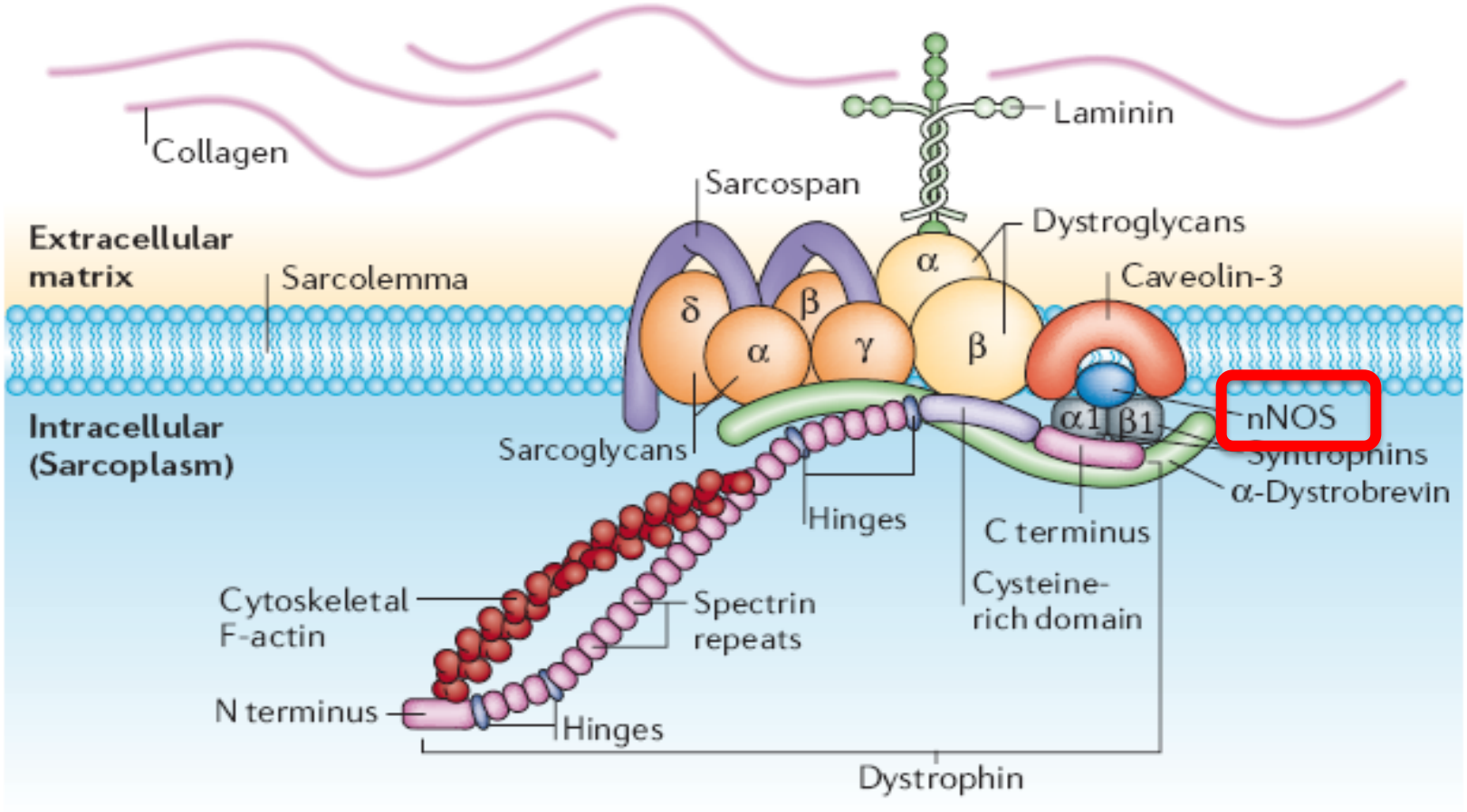
Adapted from E. Olson's lab

Patients' associations

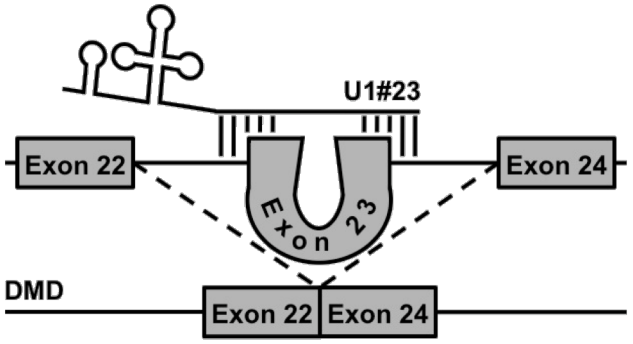
Duchenne Parent Project onlus

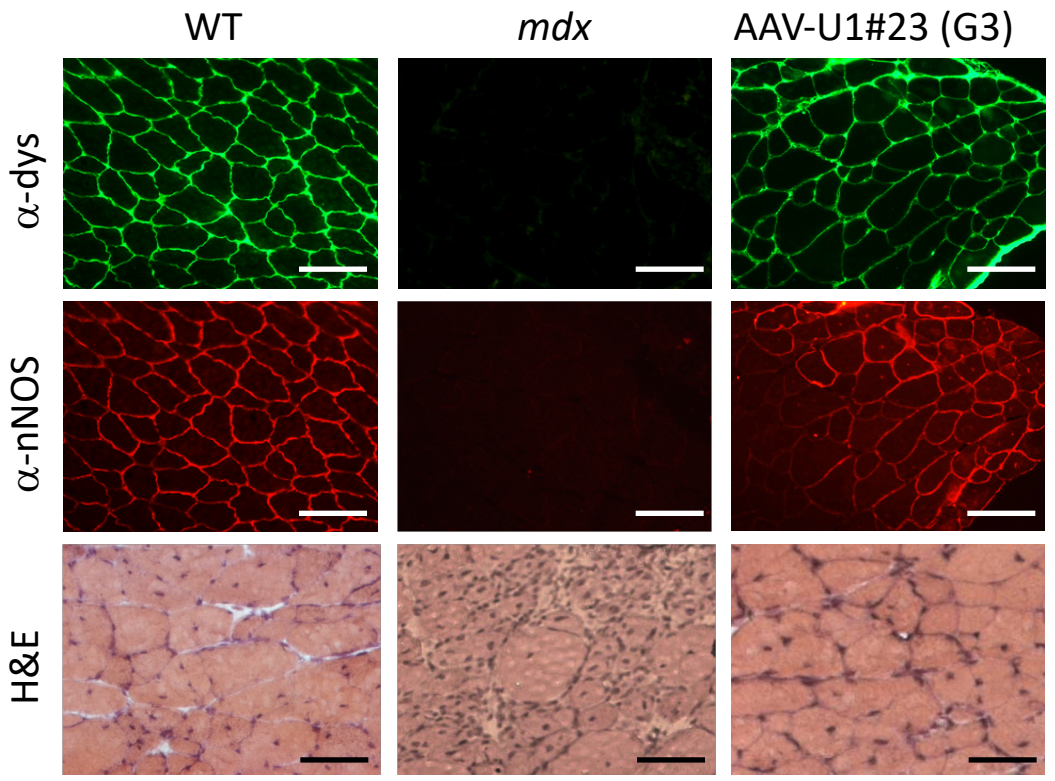
A Pasqua fai una sorpresa alla ricerca!

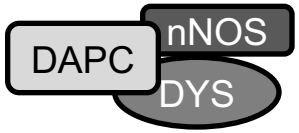
An additional role for dystrophin?



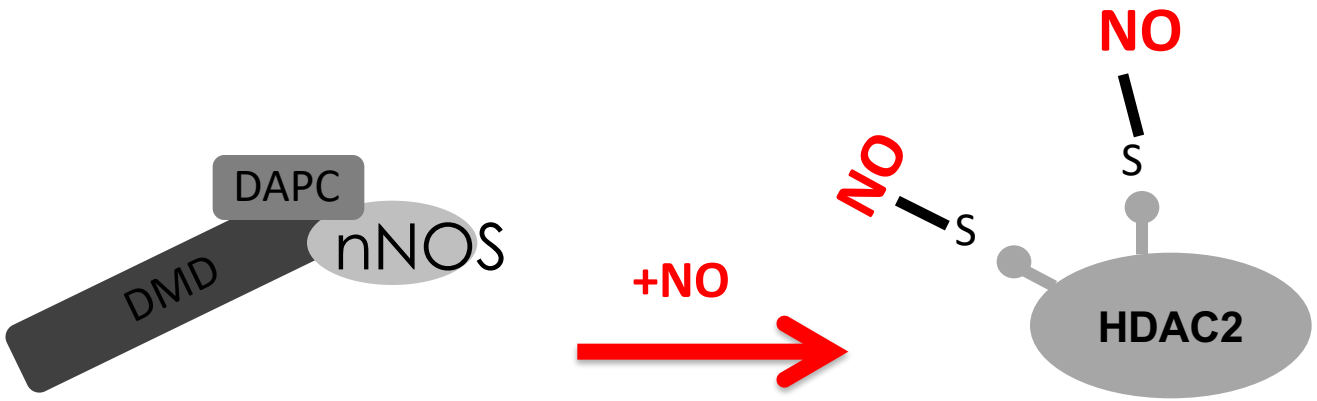
Dystrophin is required for nNOS localization



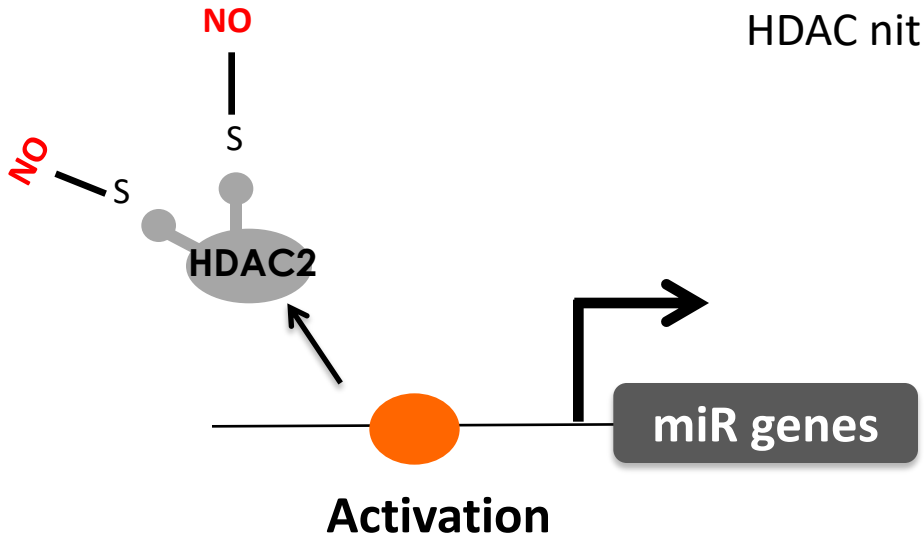




A functional link between NO and HDACs



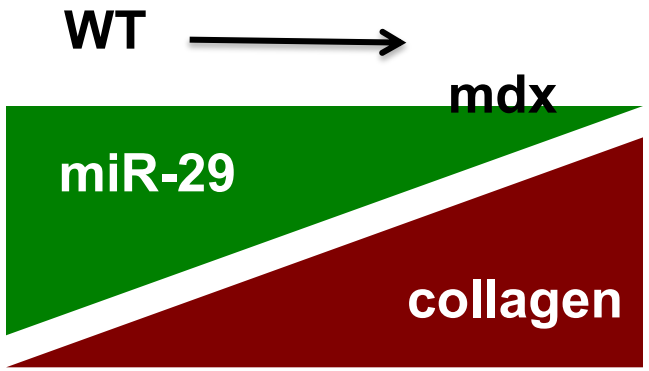
HDAC nitrosylation inactivtes the enzyme



Dystrophin activates several miRNAs that control muscle homeostasis

Cacchiarelli et al., Cell Metab. **2010**

Several miRNA targets are involved in DMD

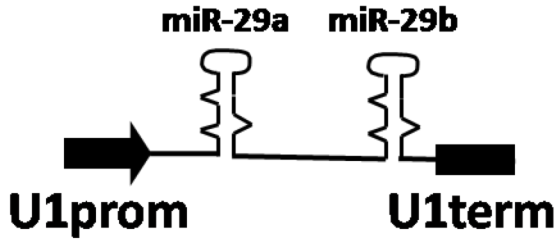


miR-29 decreases fibrosis

Duchenne no DYS ↓ miR-29 ↓ COLLAGEN/ELASTIN ↑ **FIBROSIS** ↑

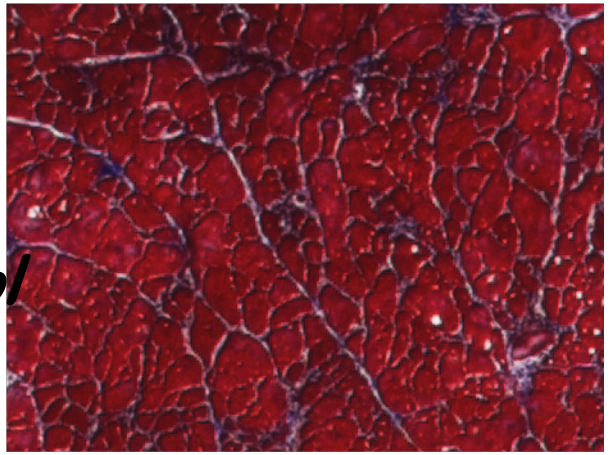
Administration of miR-29 reduces fibrosis

if administered to *mdx* muscles it reduces fibrosis

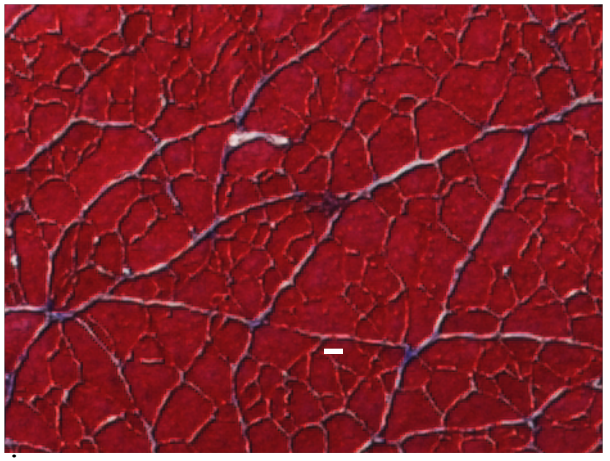


mdx

control



miR-29

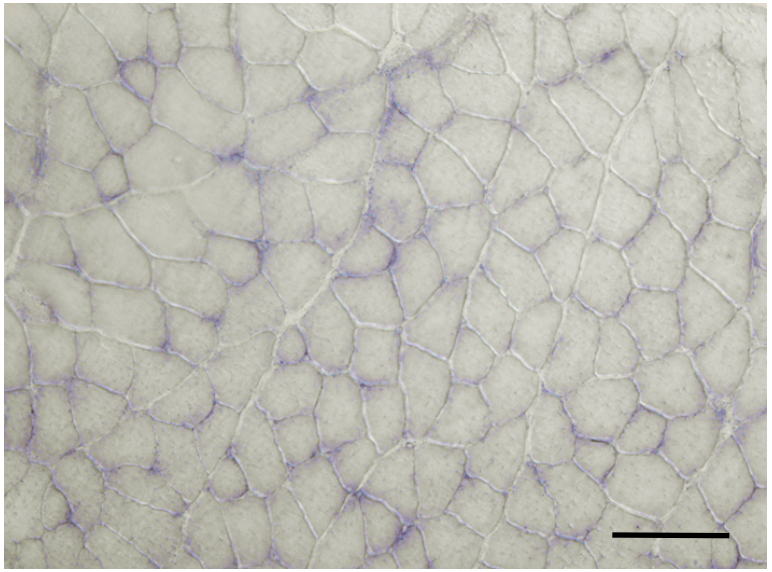


Masson's staining

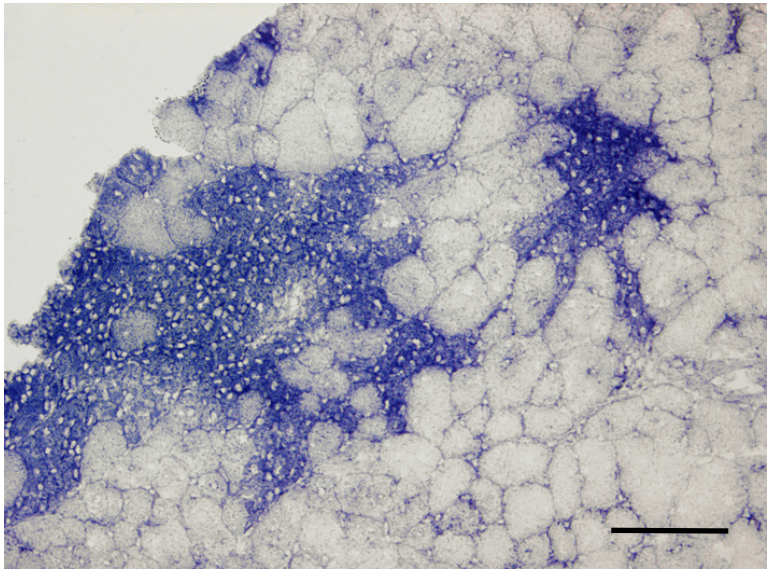
Cacchiarelli et al., Cell Metab. **2010**

miR-31 is overexpressed in DMD

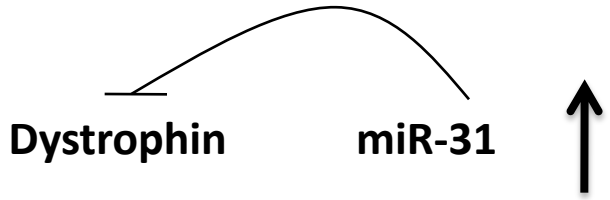
WT



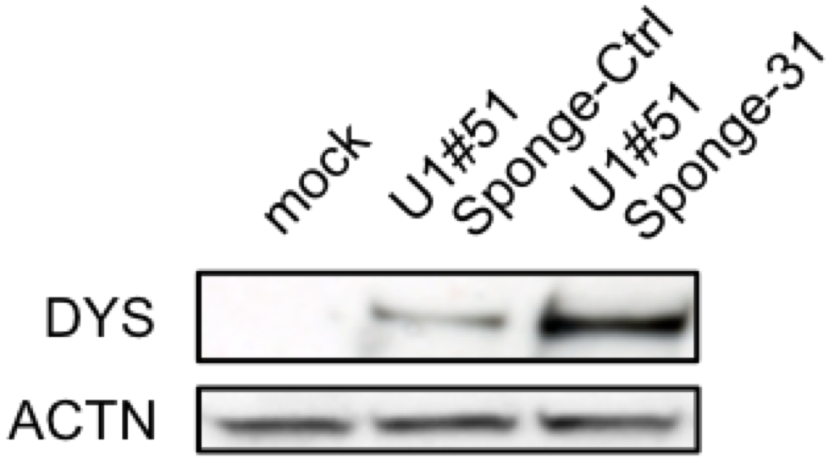
mdx



….it represses dystrophin mRNA translation !



miR-31 depletion synergizes with exon skipping…



… and possibly with all those strategies aimed to rescue dystrophin expression

Exon skipping for the cure of DMD has entered clinical trials

Synthetic oligonucleotides

van Deutekom, JC, *et al*. (2007).
Local dystrophin restoration with antisense oligonucleotide PRO051.
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