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Article

Physiopathologie de la dystrophie musculaire de Duchenne

Duchenne muscular dystrophy pathophysiology

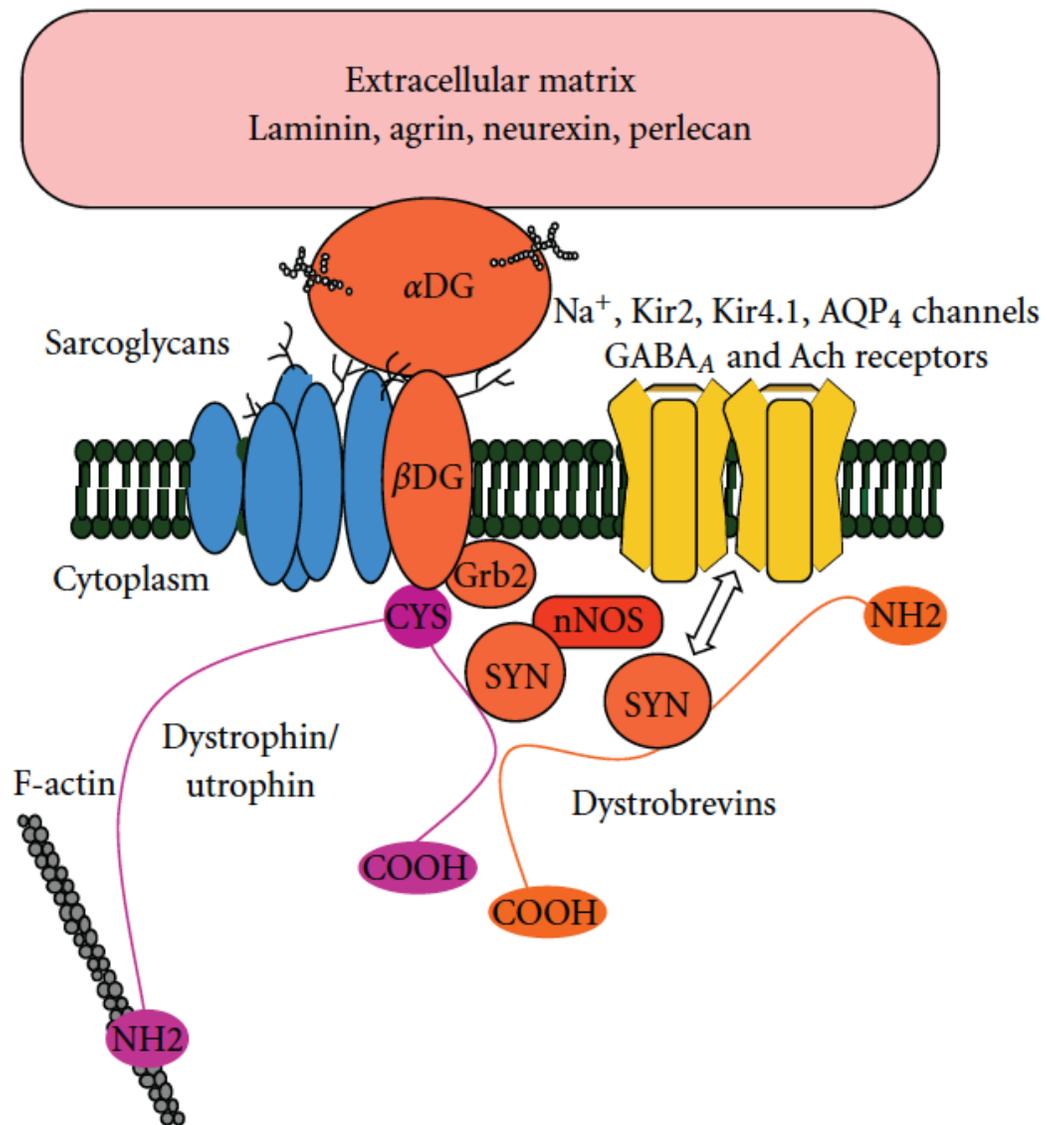
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*Centre de Référence des Maladies Neuromusculaires Nantes-Angers,
Hôtel-Dieu, 44093 Nantes cedex, France*

- 3 formes de dystrophines présentes: Dp427, Dp140, Dp70
 - Localisation différentes
 - Fonctions différentes
- Au niveau de différentes structures du SNC
 - Cervelet
 - Hippocampe
 - Cortex préfrontal
 - Cortex associatif moteur

Structures du SNC impliquées dans

- Fonctions exécutives
- Fonctions mnésiques
- Tr du spectre autistique



Mêmes partenaires qu'au niveau du muscle squelettique :

- Dystroglycane

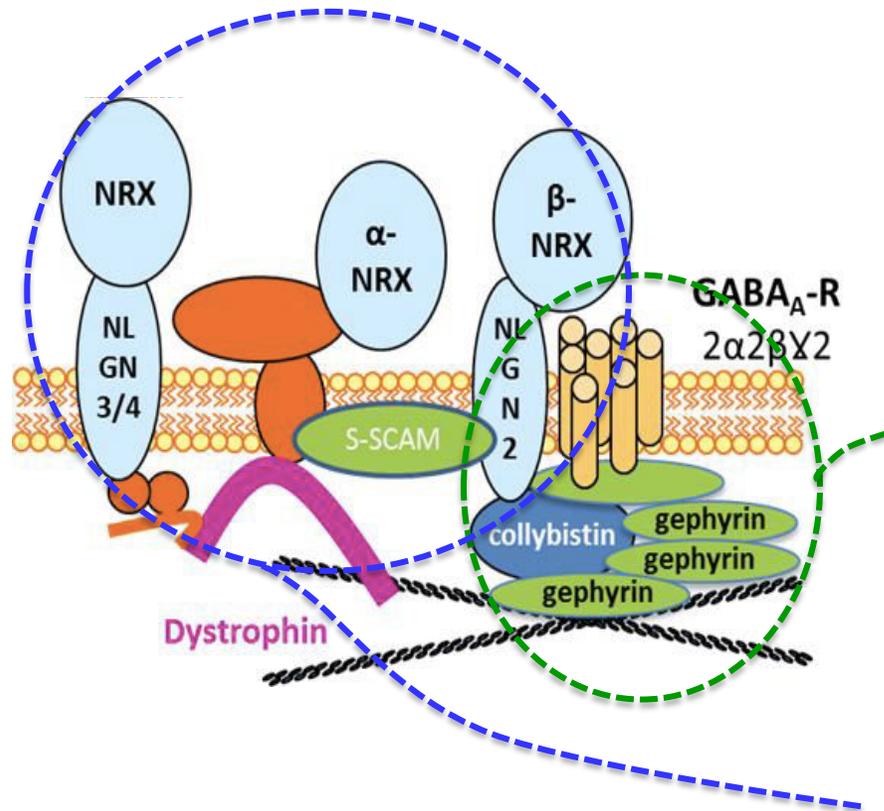
→ rôle structural: lien moléculaire entre cytosquelette et matrice extra-cellulaire

- Syntrophines, dystrobrevines, nNOS

→ Organisation moléculaire des membranes post synaptiques

- 'Signalisation' via neuroligines trans-synaptiques
- Regroupement et stabilisation de canaux ioniques et de récepteurs membranaires

Expression neuronale sélective dans les densités post synaptiques des synapses inhibitrices → stabilisation des récepteurs GABA-A



Travaux Souris mdx →

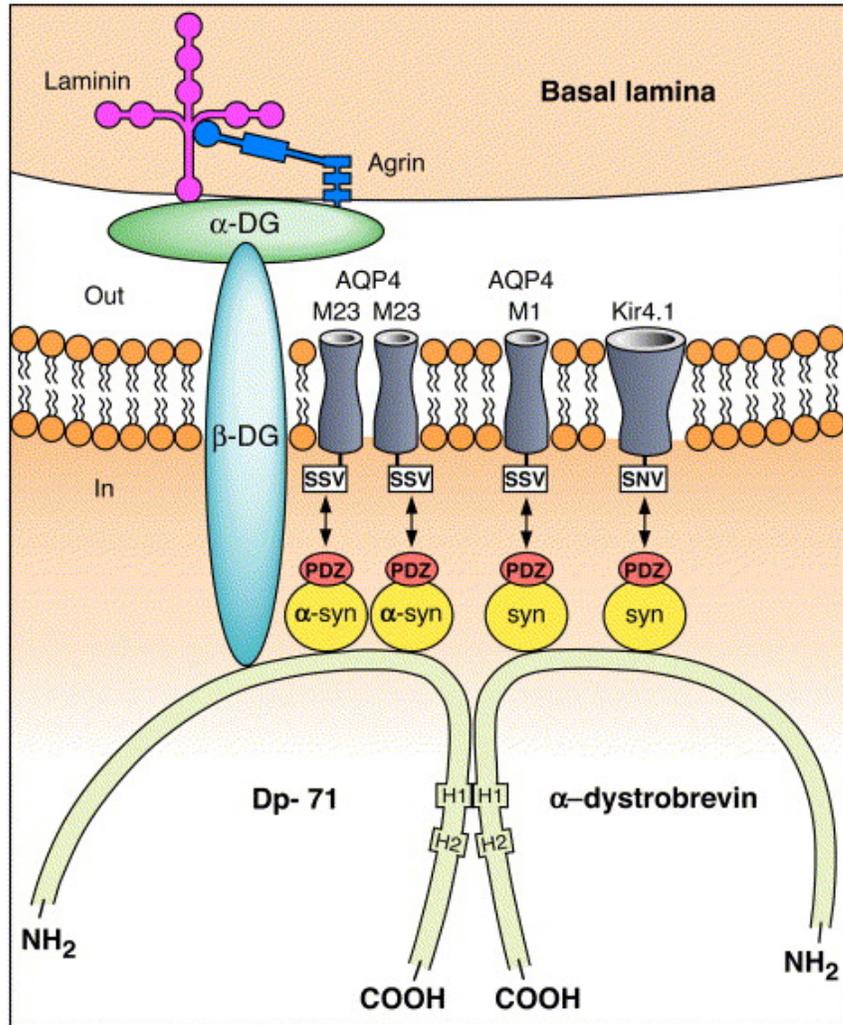
- Synapses inhibitrices neurones pyramidaux de l'hippocampe
- Interactions dystroglycane-Dp427 et protéines échafaudages associées aux GABA-A-R

→ Ancrage membranaire des GABA-A-R avec actine du cytosquelette sous-synaptique via géphyrine et collybistin pendant synaptogenèse

→ Interaction avec complexe trans-synaptique des Neuroligines (NLGN) / Neurexine (NRX)

→ Stabilisation/modulation clustering GABA-A-R (NLGN2 via SCAM, NLGN3/4 via les syntrophines, α -NRX via le dystroglycane)

→ Dp427 impliquée dans la fonction GABAergique et dans la plasticité synaptique du cerveau adulte



Dp71 + complexe DAPC dans les pieds des astrocytes périvasculaires:

→ Interactions entre canaux AQP4 et α -syntrophines liées au domaine C-terminal de la Dp71

→ Rôle dans l'ancrage des canaux AQP4 et Kir4.1 ?

→ Actions sur homéostasie hydrique et perméabilité vasculaire ?

→ *Altération de la maturation et de la plasticité des synapses glutamatergiques*

→ Dp71 impliquée à la fois dans des *fonctions astrocytaires et neuronales*

- Dp427 & Dp71 → structures SNC impliquées dans processus cognitifs et comportementaux
 - Dp427 & Dp71 → organisation moléculaire des membranes post synaptiques
 - Agrégation (clustering) et ancrage de canaux ioniques
 - Dp427 → réc. GABA-A dans les synapses des neurones pyramidaux
 - Dp71 → canaux AQP4 et Kir4.1 à l'interface glio-vasculaire
 - maturation et plasticité des synapses glutamates
-
- Rôle très probable au cours du développement et de la plasticité fonctionnelle du cerveau adulte
 - Conséquences sur processus cognitifs et dans d'autres fonctions comportementales (réactivité émotionnelle, capacités motrices, comportement social)

Les progrès depuis 25 ans

- **La prise en charge multidisciplinaire**

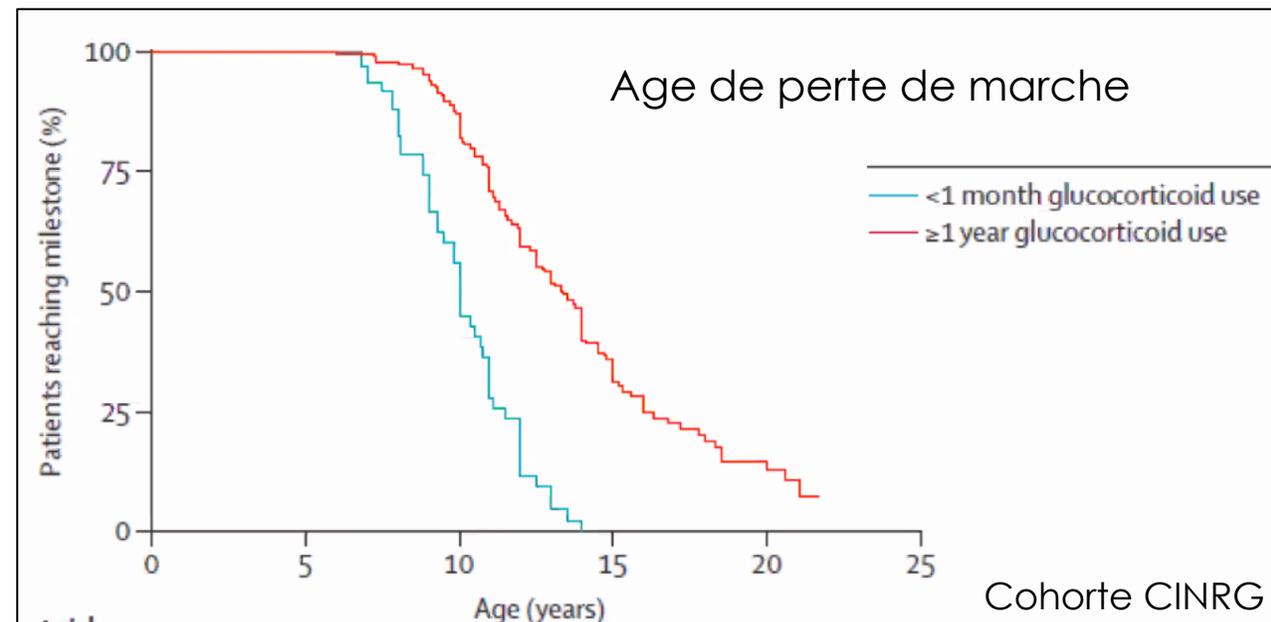
- Suivi bisannuel
- Ventilation non invasive, trachéotomie
- Chirurgie de scoliose
- Kinésithérapie : prévention des rétractions ++

- **Les corticoïdes**

→ retardent la perte de marche de 18 à 24 mois

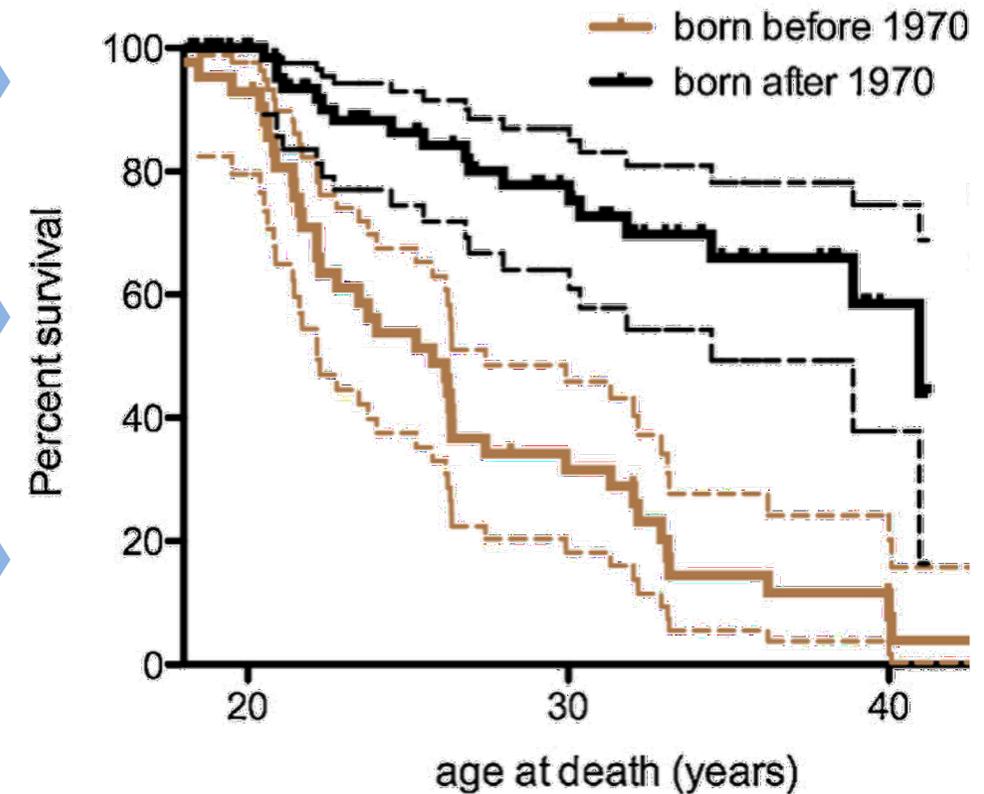
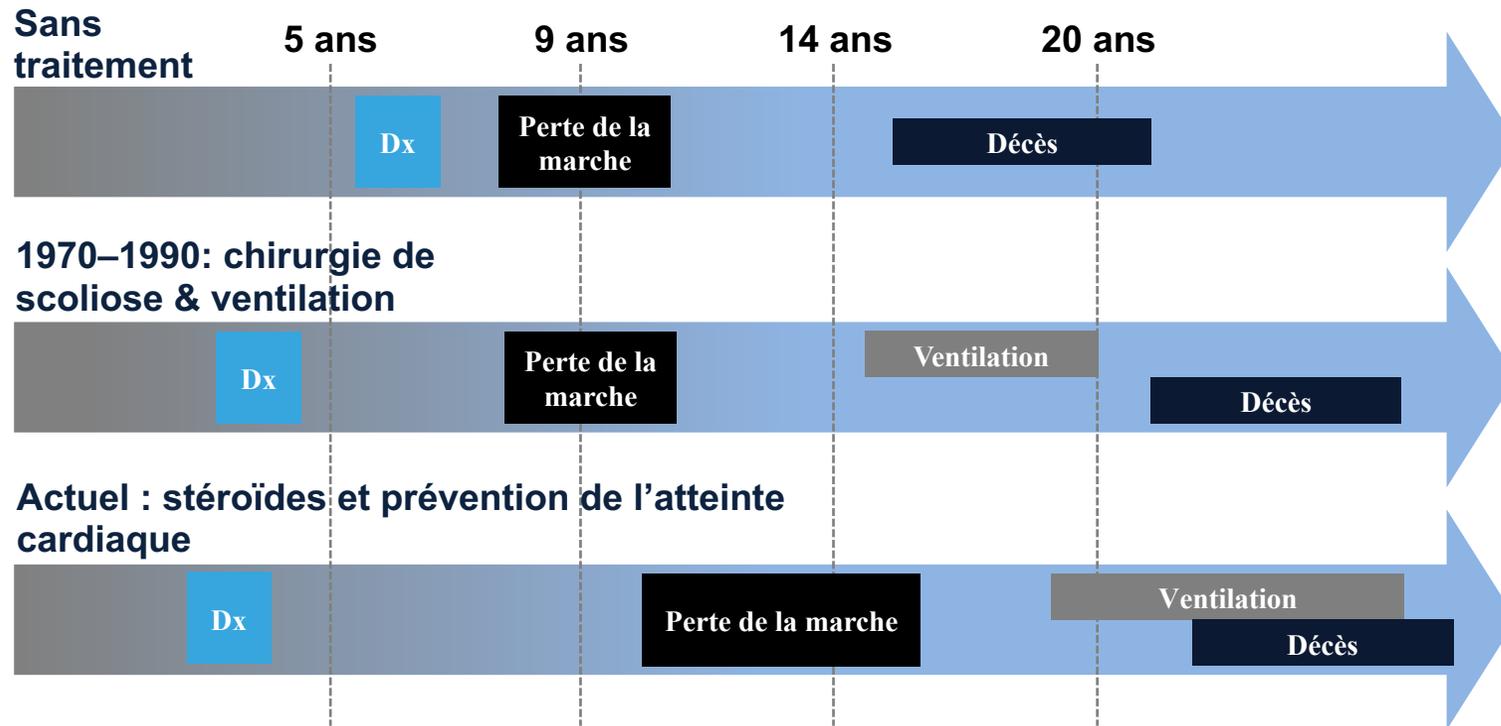
- **Le périndopril** (Duboc et al, JACC, 2005)

→ retarde l'apparition et ralentit l'évolution de l'atteinte cardiaque



L'espérance de vie atteint maintenant 35-40 ans !

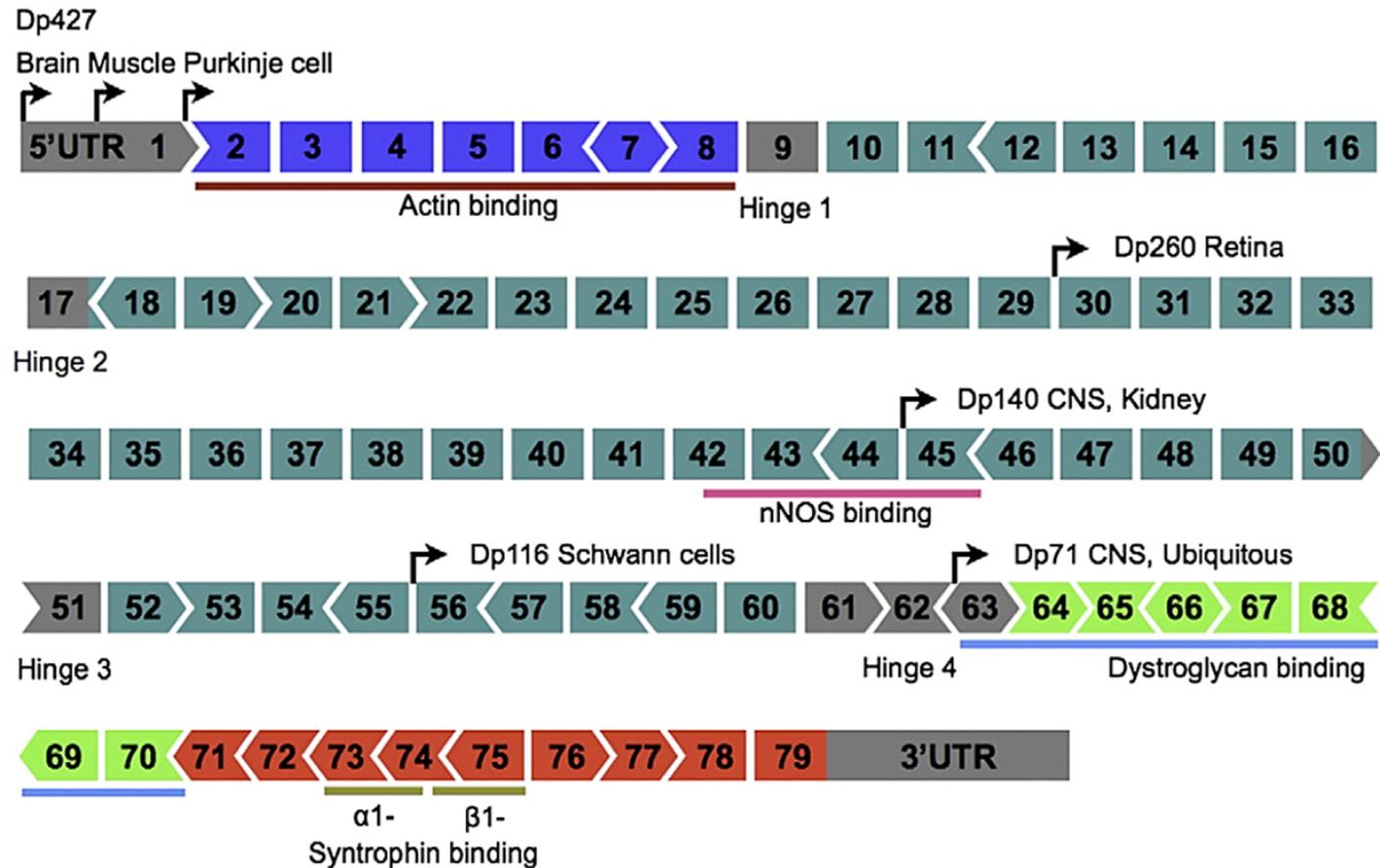
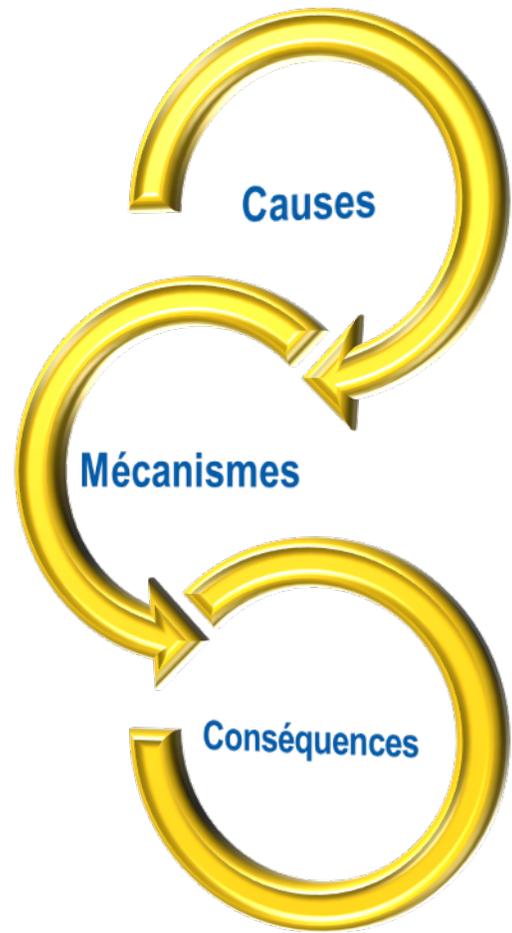
Les progrès depuis 25 ans

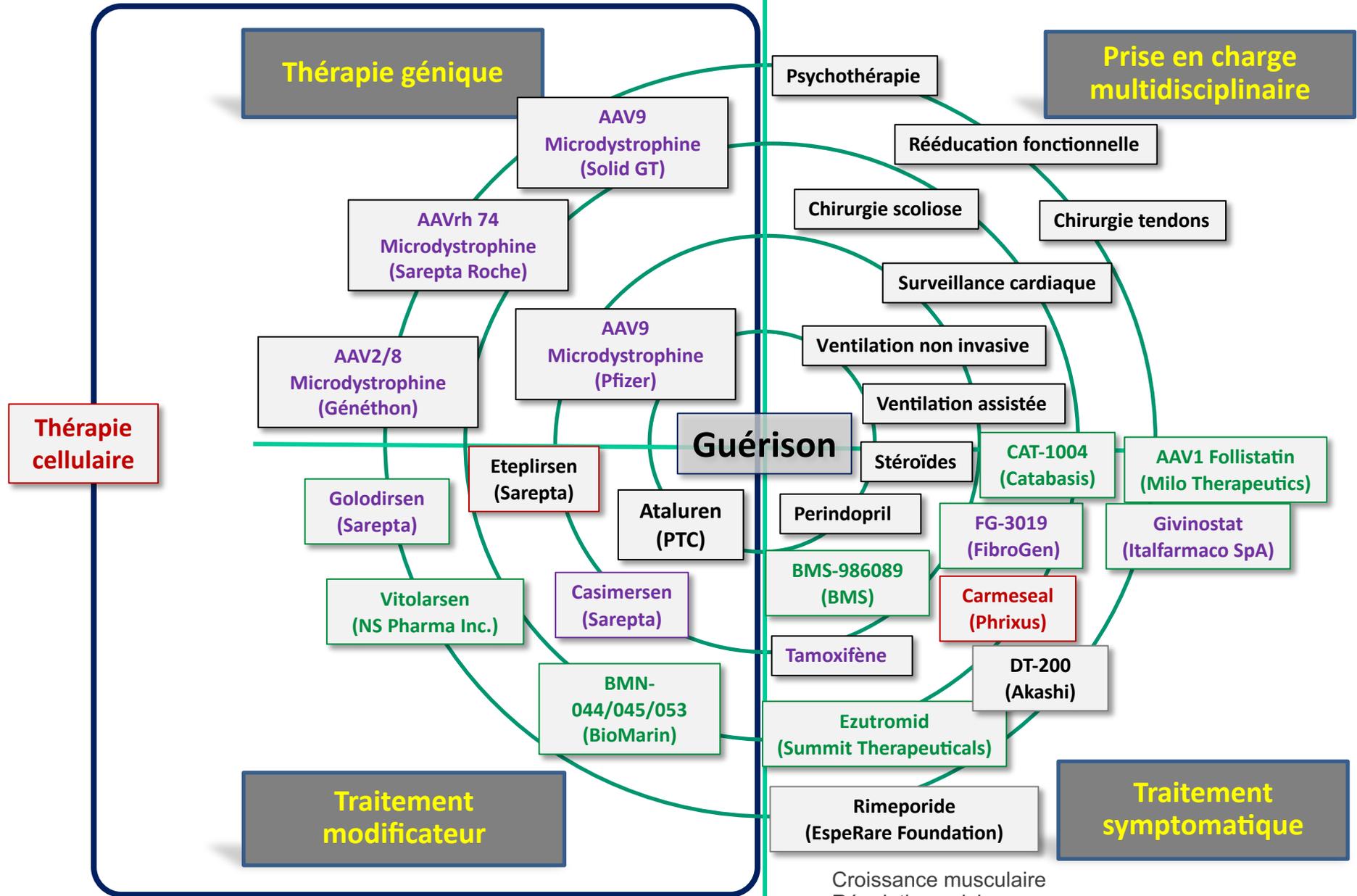
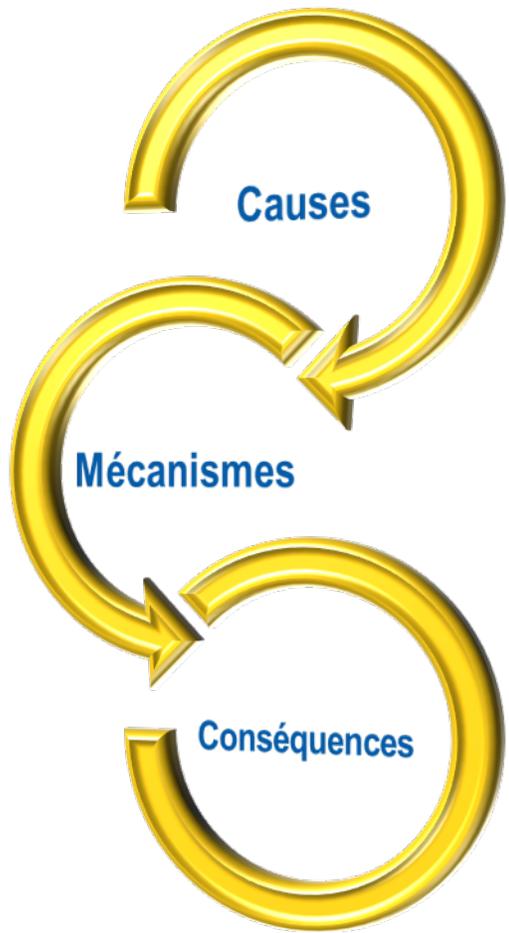


Dx = diagnostic

1. Bushby K, Connor E. *Clin Investig (Lond)* 2011;1:1217–35.
2. McDonald CM *et al. Muscle Nerve* 2013;48:32–54.
3. Kiény P *et al. Ann Phys Rehab Med* 2013;56:443-454

Où agir ?



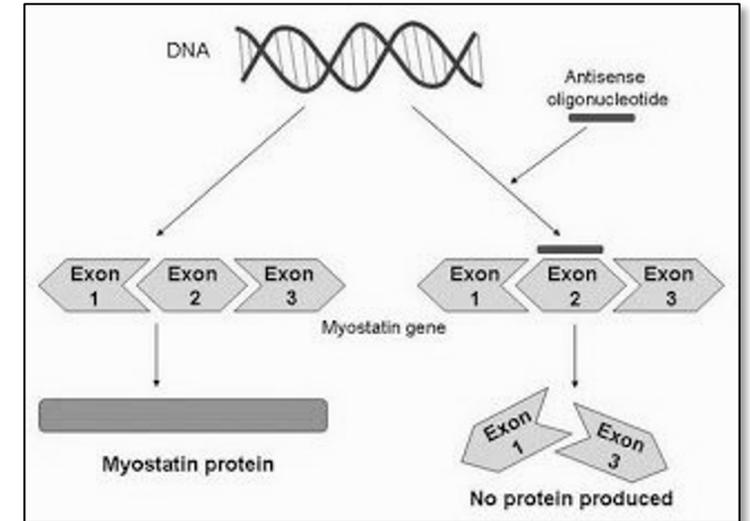
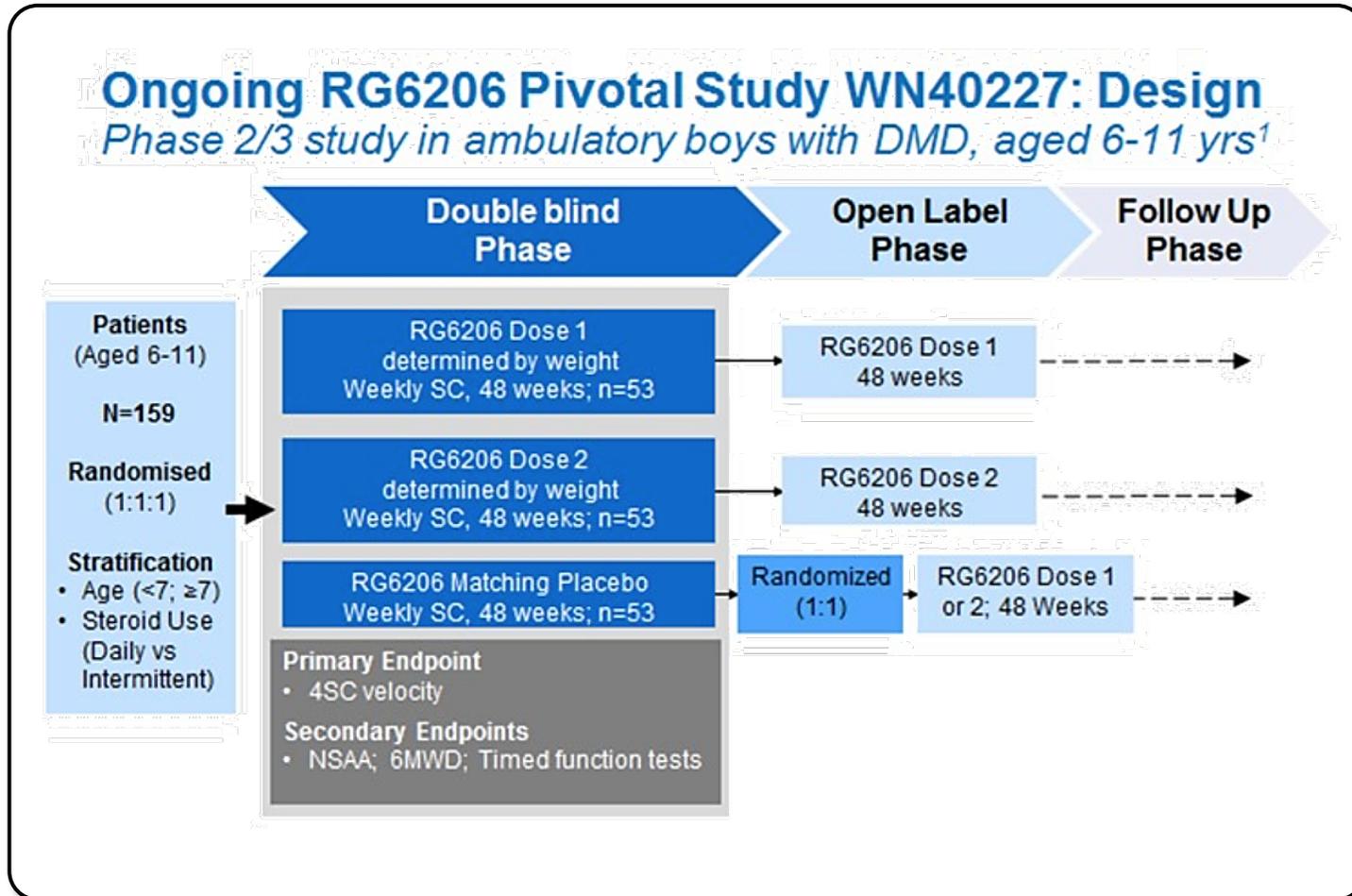


Traitements / gène & expression de la dystrophine

- Croissance musculaire
- Régulation calcique
- Anti-inflammatoires / Anti-fibrose
- Facilitateur mitochondriaux
- Renforcement tissu cardiaque

Préclinique
Phase I
Phase II
Phase III

Thérapie pharmacologique : *en renforçant les fibres musculaires / anti-myostatine*



Thérapie d'expression de gène : *en sautant un codon stop*

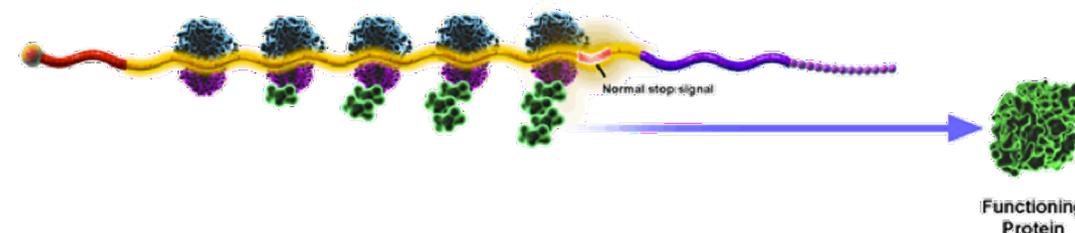
Ataluren

J Comp Eff Res. 2020 Oct;9(14):973-984

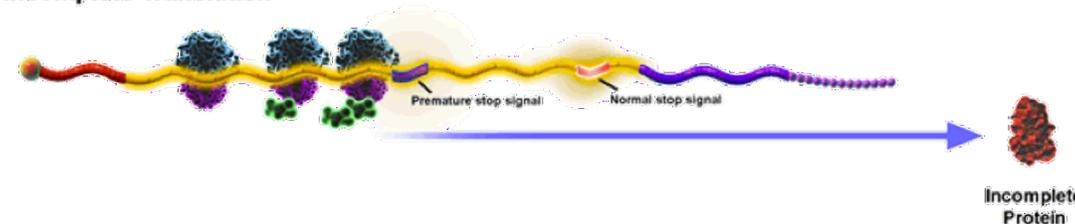
Meta-analyses of ataluren randomized controlled trials in nonsense mutation Duchenne muscular dystrophy

Craig Campbell^{*.1} , Richard J Barohn², Enrico Bertini³, Brigitte Chabrol⁴, Giacomo Pietro Comi⁵, Basil T Darras⁶, Richard S Finkel^{7,8}, Kevin M Flanigan⁹, Nathalie Goemans¹⁰, Susan T Iannaccone¹¹, Kristi J Jones¹², Janbernd Kirschner¹³, Jean K Mah¹⁴, Katherine D Mathews¹⁵, Craig M McDonald¹⁶, Eugenio Mercuri¹⁷, Yoram Nevo¹⁸, Yann Péréon¹⁹, J Ben Renfro²⁰, Monique M Ryan²¹, Jacinda B Sampson²², Ulrike Schara²³, Thomas Sejersen²⁴, Kathryn Selby²⁵, Már Tulinius²⁶, Juan J Vilchez²⁷, Thomas Voit²⁸, Lee-Jen Wei²⁹, Brenda L Wong³⁰, Gary Elfring³¹, Marcio Souza³¹, Joseph McIntosh³¹, Panayiota Trifillis³¹, Stuart W Peltz³¹, Francesco Muntoni²⁸, on behalf of the PTC124-GD-007-DMD Study Group[‡], ACT DMD Study Group[‡] & the Clinical Evaluator Training Groups[‡]

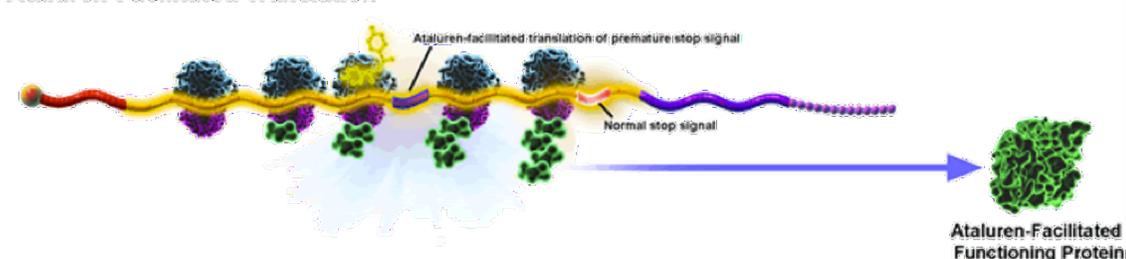
Normal Translation



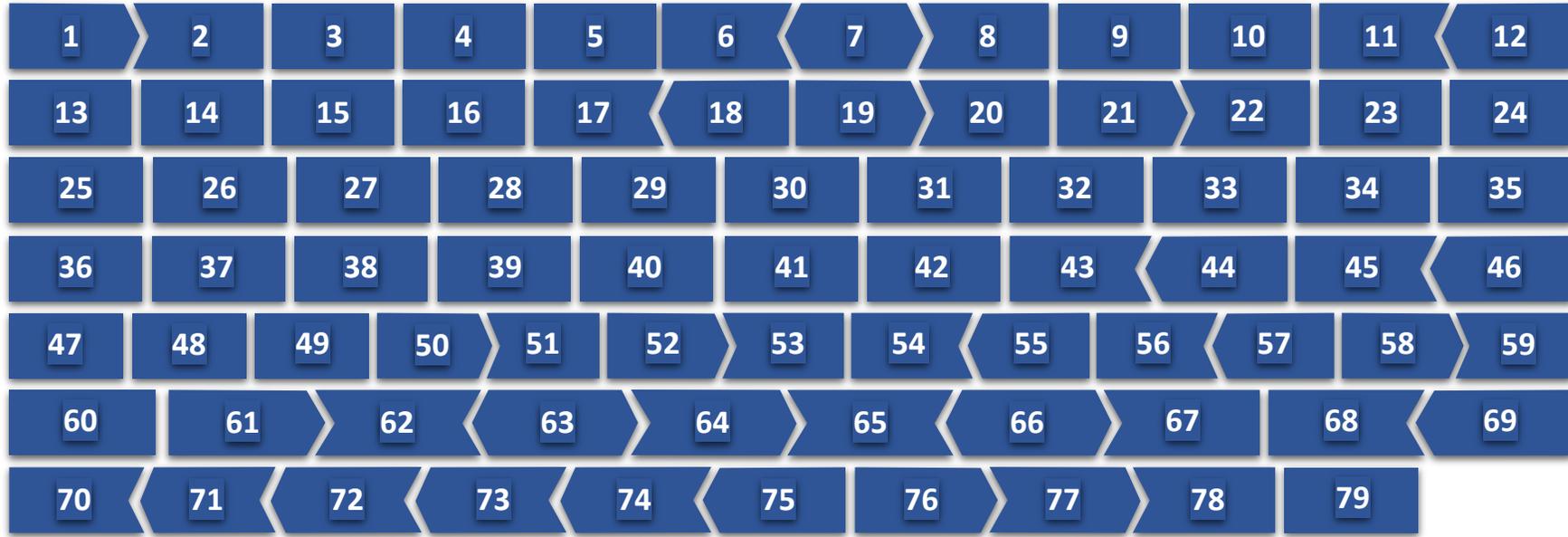
Incomplete Translation



Ataluren-Facilitated Translation



Thérapie d'expression de gène : *par du saut d'exons*



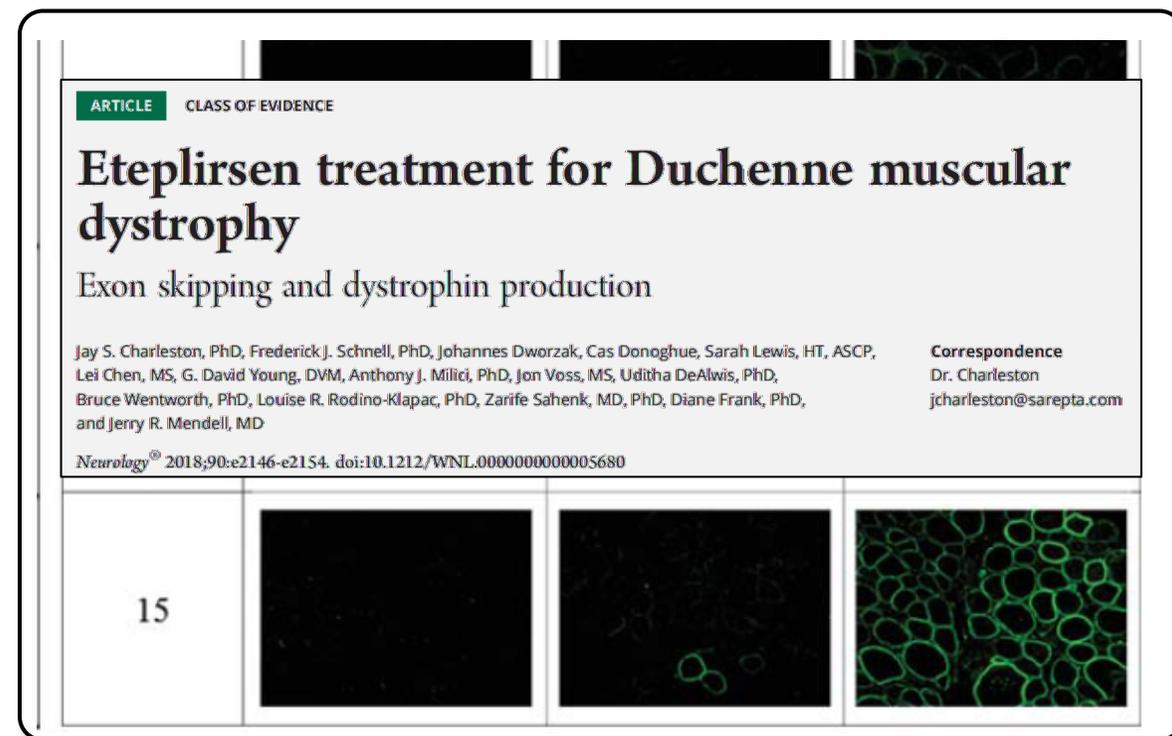
Dystrophie Musculaire de Duchenne → *Dystrophie Musculaire de Becker*

- Conservation du cadre de lecture → BMD
- Décalage du cadre de lecture → DMD

Thérapie d'expression de gène : *par du saut d'exons*

Eteplirsén

- Eteplirsén (exon 51, Exondys 51[®])
- Golodirsén (exon 53, Vyondys 53[®])
- Casimersén (exon 45, essai ESSENCE)
- Vitolarsén (exon 53)
- ~~Drisapersén (exon 51)~~
- ~~Sudovirsén (exon 51)~~
- ...



Thérapie génique :

en remplaçant le gène avec des AAV

Full-length dystrophin (Hoffman et al 1987)



Δ DysM3 (Yuasa et al 1997)



Δ 3990 (Wang et al 2000)



Δ R4-23/ Δ C (Harper et al 2002)(also called Δ CS1, MD1, H2 μ Dys)



μ Dys-5R (Hakim et al 2017)



Duan, Mol Ther 2018; 26:2337–2356

Phénotype DMD (myopathie de Duchenne) \rightarrow Phénotype BMD (myopathie de Becker)

A Study to Evaluate the Safety and Tolerability of PF-06939926 Gene Therapy in DMD

Study Description

Go to

Brief Summary:

This is a first-in-human/first-in-patient, multi-center, open-label, non-randomized, ascending dose, safety and tolerability study of a single intravenous infusion of PF-06939926 in ambulatory subjects with Duchenne muscular dystrophy (DMD). Other objectives include measurement of dystrophin expression and distribution, and assessments of muscle strength, quality, and function.

Two dose cohorts are planned with up to 6 subjects for each. In order to mitigate unanticipated risks to subject safety, enrollment will be staggered within and between the two cohorts and will include a formal review by an external data monitoring committee (E-DMC) prior to dose progression.

ARTICLE

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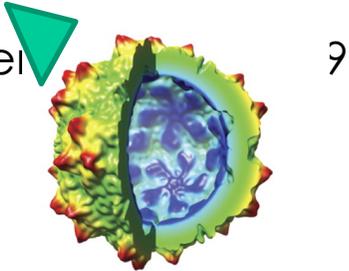
Long-term microdystrophin gene therapy is effective in a canine model of Duchenne muscular dystrophy

Caroline Le Guiner^{1,2}, Laurent Servais³, Marie Montus², Thibaut Larcher⁴, Bodvaël Fraysse¹, Sophie Moullec⁵, Marine Allais¹, Virginie François¹, Maeva Dutilleul⁴, Alberto Malerba⁶, Taeyoung Koo⁶, Jean-Laurent Thibaut^{7,8}, Béatrice Matot⁷, Marie Devaux¹, Johanne Le Duff¹, Jack-Yves Deschamps⁵, Inès Barthelemy^{8,9}, Stéphane Blot^{8,9}, Isabelle Testault¹⁰, Karim Wahbi¹¹, Stéphane Ederhy¹², Samia Martin², Philippe Veron², Christophe Georger², Takis Athanasopoulos^{6,13,†}, Carole Masurier², Federico Mingozzi², Pierre Carlier⁷, Bernard Gjata², Jean-Yves Hogrel^{1,4}, Oumeya Adjali¹, Fulvio Mavilio², Thomas Voit^{15,*}, Philippe Moullier^{1,16,*} & George Dickson^{6,*}

Thérapie génique :

en remplaçant le gène avec des AAV

4 essais internationaux dont 3 en France

- Pfizer (Nantes, Necker), AAV9
- Sarepta Roche (Necker), AAVrh74
- Généthon (I-motion, Strasbourg...), AAV2/8
- Solid Biosciences  9

AAV9 cross section

A PHASE 3, MULTICENTER, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY TO EVALUATE THE SAFETY AND EFFICACY OF PF-06939926 FOR THE TREATMENT OF DUCHENNE MUSCULAR DYSTROPHY

Investigational Product Number:	PF-06939926
Investigational Product Name:	Not Applicable
United States (US) Investigational New Drug (IND) Number:	17598
European Clinical Trials Database (EudraCT) Number:	Not Available
Protocol Number:	C3391003
Phase:	3
Short Title:	A phase 3, randomized, double-blind, placebo-controlled study of PF-06939926 in DMD.

Thérapie génique :

en réparant le gène par CRISPR/Cas9

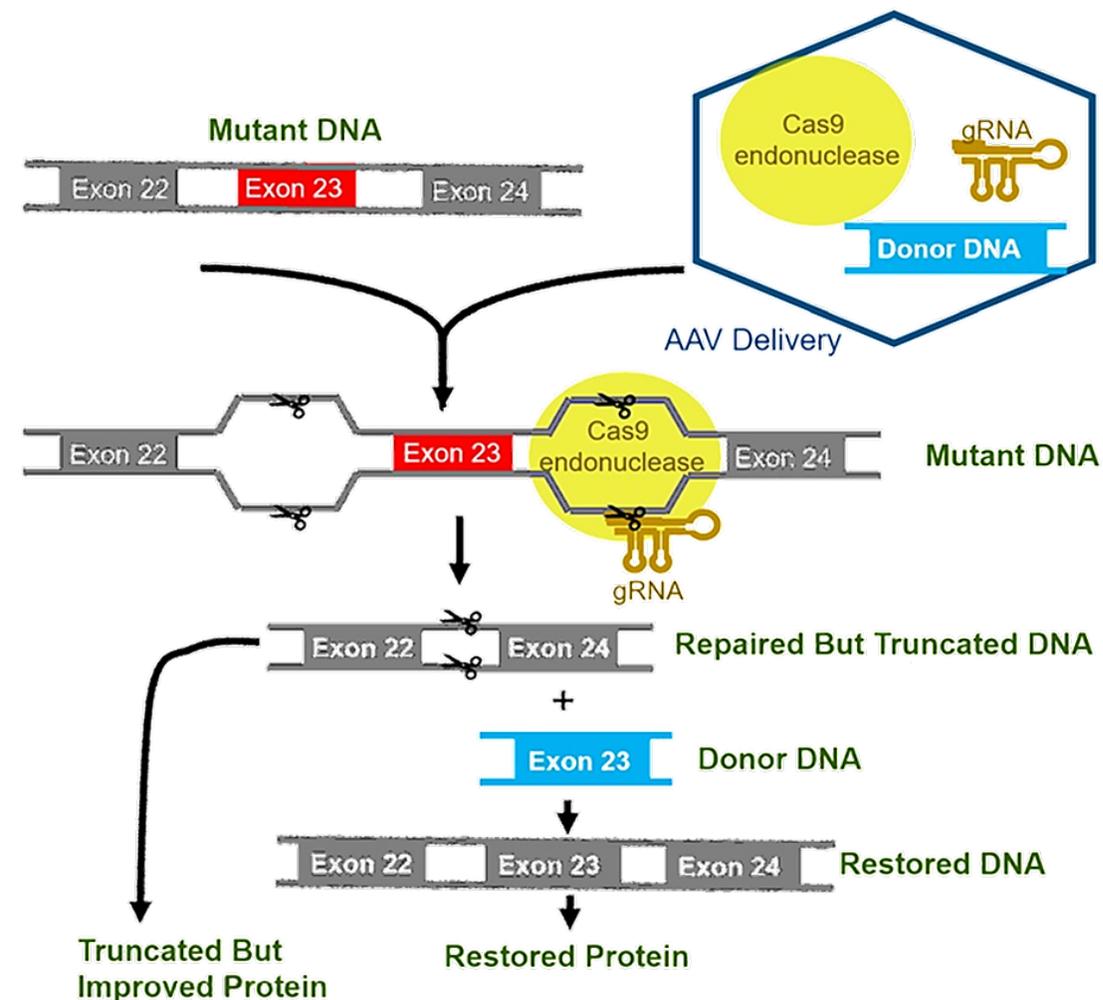
The CRISPR/Cas system for gene editing

The CRISPR/Cas9 gene editing system consists of two components: the Cas9 endonuclease and a guide RNA (gRNA), which is composed of a target spacer region for DNA binding and a scaffold region for binding to Cas9.

The gRNA spacer sequence is designed to be homologous to a target site in the genome, which must have a 3' protospacer adjacent motif (PAM) sequence.

gRNA targeting results in Cas9 creating a double-stranded break (DSB) in the DNA 3 bp upstream of the PAM.

The DSB can be repaired either through the cells endogenous repair, non-homologous end joining (NHEJ), which can sometimes result in small insertions or deletions (indels), or through homology-directed repair (HDR) to incorporate new DNA by addition of template DNA containing homology arms.



Thérapie génique : *en réparant le gène par CRISPr/Cas9*

Table 1. Summary of CRISPR/Cas9 approaches for NMDs

Approach	NMD	Model	References
NHEJ-mediated single exon deletion	DMD	Cells, mice	37, 50, 66, 80, 84, 85, 97, 98, 101, 122
NHEJ-mediated multi-exon deletion	DMD	Cells, mice	15, 16, 36, 55, 69, 84, 85, 101, 110, 135, 137, 138
NHEJ-mediated single cut	DMD	Cells, mice, dog	5, 6, 66, 76, 81, 85, 90, 93, 141
NHEJ-mediated duplication deletion	DMD	Cells	71, 133
NHEJ-mediated repeat region deletion	DM1	Cells, mice	3, 32, 105, 131
NHEJ-mediated intronic deletion	MDC1A	Mice	60
	UCMD	Cells	17
HDR-mediated point mutation repair	DMD	Cells, mice	15, 74, 82, 141, 142
	LGMD2B, 2D	Cells	126
HDR-mediated exon replacement	DMD	Cells	76
HDR-mediated polyA insertion	DM1	Cells	131
Base editing	DMD	Cells, mice	112, 139
dCas9 activation	DMD	Cells, mice	78, 102, 133
	MDC1A	Mice	59
dCas9 inactivation	FSHD	Cells	52
dCas9 interference	DM1	Cells, mice	13, 104



Available online at www.sciencedirect.com



Neuromuscular Disorders 21 (2011) 4–12



www.elsevier.com/locate/nmd

Review

Stem cells to treat muscular dystrophies – Where are we?

Jinhong Meng, Francesco Muntoni, Jennifer E. Morgan *

The Dubowitz Neuromuscular Centre, UCL Institute of Child Health, 30 Guilford Street, London WC1N 1EH, United Kingdom

Received 1 June 2010; received in revised form 13 September 2010; accepted 8 October 2010

Abstract

The muscular dystrophies are inherited disorders characterised by progressive muscle wasting and weakness. Stem cell therapy is considered to be one of the most promising strategies for treating muscular dystrophies. In this review, we first examine the evidence that a stem cell could be used to treat muscular dystrophies, and then discuss the criteria that an ideal stem cell should meet. We also highlight the importance of standard operation procedures to be followed for ensuring the consistent and reproducible efficacy of a particular stem cell. While at the moment the scientific community is looking for an ideal stem cell to treat muscular dystrophies, it is clear that in order for this field to benefit from therapeutic stem cell applications, additional careful investigations are required.

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Keywords: Stem cell; Muscular dystrophies; Myoblast; Muscle-derived stem cell; Mesoangioblast; AC133+ cell; ES cell; Induced pluripotent stem cell; Satellite cell

Thérapie cellulaire

2510 Rouger et al
AJP November 2011, Vol. 179, No. 5

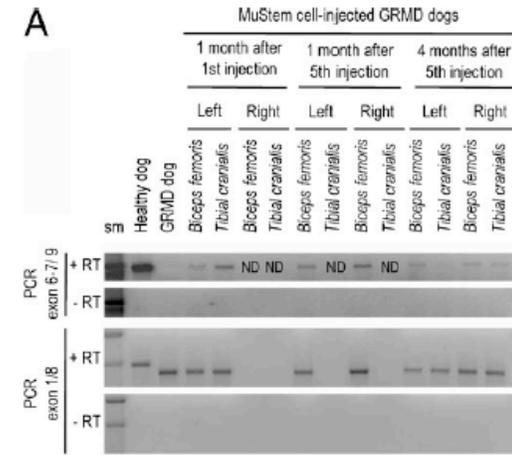
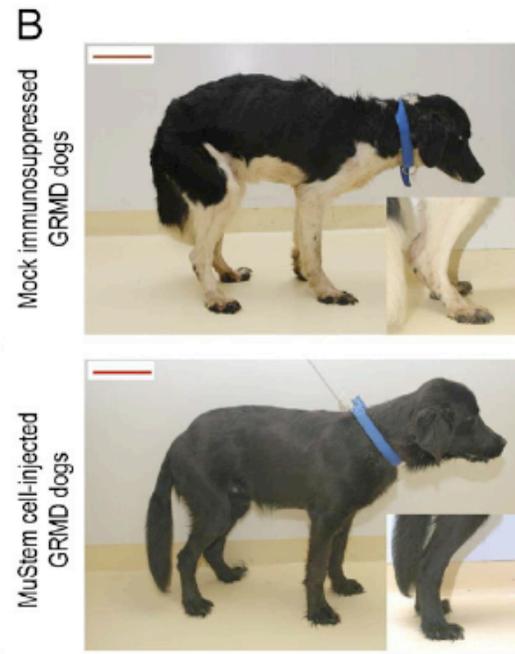
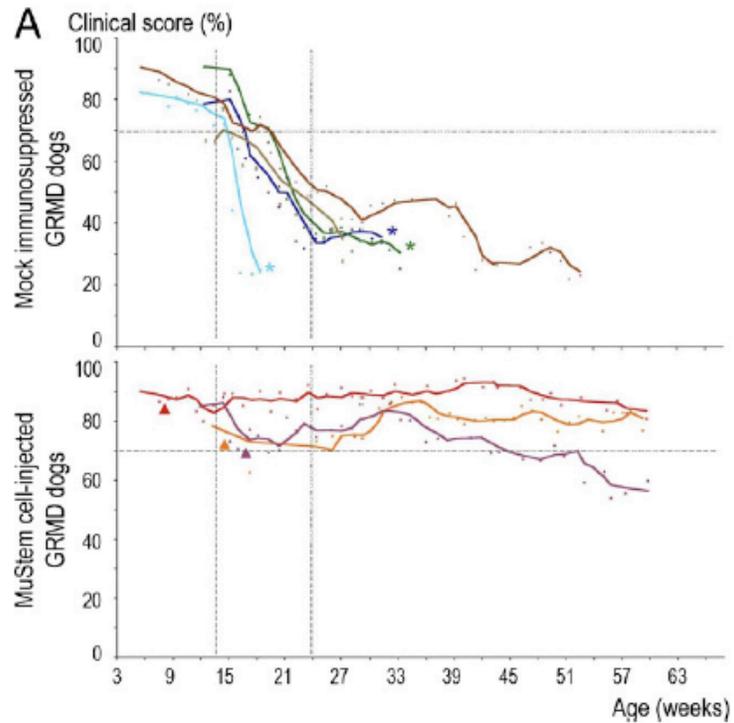
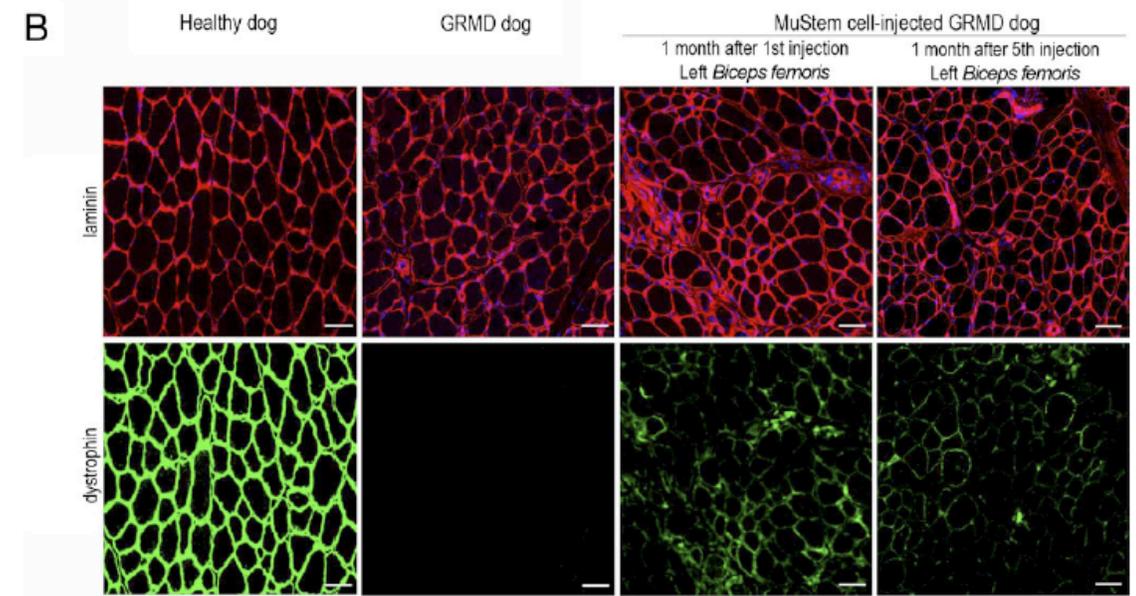


Figure 7. Dystrophin expression after systemic delivery of MuStem cells. **A:** RT-PCR analysis revealed the presence of wild-type dystrophin mRNA (PCR "exon 6/7 to exon 9") on muscle biopsies collected at various time points after the first cell injection. The PCR "exon 1/exon 3" was shown as an internal control. **B:** Immunolabeling for laminin (red) and dystrophin (green) showed the presence of numerous scattered fibers with dystrophin expression in the whole muscle section, at the different time points of the protocol ($n = 3$ per muscle and time point). Scale bar = 50 μm . ND, non-determined; sm, size markers.



Des règles de bonne pratique

Maladies rares ... mais nombreuses

Savoir y penser - culture du doute

→ Centres de Référence Maladies Neuromusculaires

