Therapeutic approach design for Duchenne muscular dystrophy

Autologous *ex vivo* cell therapy and *in vivo* gene therapy

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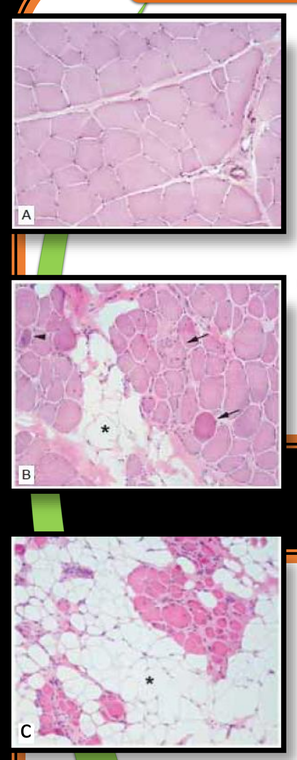
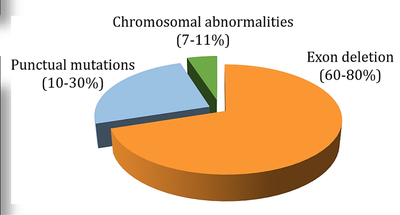
INTRODUCTION



◀ **Figure 1. Muscular degeneration in DMD**[6]. A) Normal muscle histology. B) Early alterations such as necrosis. C) Late stage of myopathy with adipocyte infiltration and fibrotic tissue. *, adipocytes; arrow, dystrophic fibers.

Duchenne muscular dystrophy (DMD)[1] is the most severe and common myopathy, affecting one in every 3,500 newborn males.

DMD is a recessive lethal X-linked disorder caused by the **absence of dystrophin** in muscle fibers. The main function of dystrophin is to stabilize myofibers during muscle contractions. DMD is characterized by progressive muscle degeneration, which is replaced with adipose and fibrotic connective tissue (Fig.1). Central nervous system (CNS) and bones are also affected. The clinical progression of DMD is reflected in Figure 2.

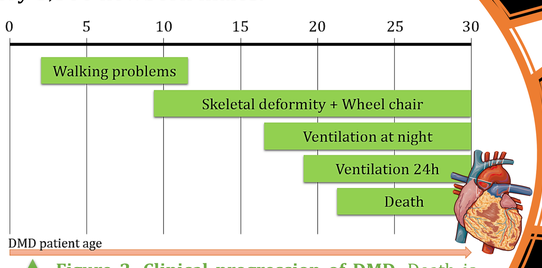


◀ **Figure 3. Genetic causes of DMD**[1]

This disorder is caused by an alteration in the frameshift of the dystrophin gene whereby result in a premature stop codon, which could be generated by these three main categories (Fig.3).

The size of the dystrophin gene is 2'4Mb, the biggest human gene. It contains 79 exons that code for **14kb mRNA** and has 7 isoforms[1].

For these reasons, the treatment of DMD with gene therapy is difficult and still a challenge nowadays.



▲ **Figure 2. Clinical progression of DMD.** Death is due to a cardiorespiratory failure.

AIM

Due to the severity and high incidence of Duchenne disease, the aim of this project is to determinate the optimal therapeutic approach restoring muscle function and ameliorating symptoms and life expectancy.

STATE OF THE ART

There are currently **no curative treatments** for this disease, but different ameliorating therapeutic approaches are being studied. Table 1 shows current treatments and approaches for DMD[1].

▼ **Table 1. Therapeutic state of the art of DMD**[1]

	PHARMACOLOGY						GENE THERAPY
	OTHER APPROACHES			DYSTROPHIN RESTORATION APPROACHES			
	Corticosteroids (prednisone)	Utrophin up regulators	Miostatin blockers	Stop codon read-through drugs	Antisense oligonucleotides	Nucleases (ZFN, TALEN, CRISPR)	
Function	Prolonged ambulation for about 2 years.	↑ utrophin could slow down DMD development	Muscle strength ↑ with hypertrophy and hyperplasia.	Introduction an amino acid at the premature stop codon to continue the mRNA translation.	Exon skipping due to the interaction with splicing signals in pre-mRNA.	Restoration of the dystrophin reading frame by micro-deletion o micro-insertion.	There are lots of strategies to tackle DMD, either using viral vectors or without them.
Problems	Side effects such as weight gain and bone demineralization.	Drugs cannot ↑ utrophin sufficiently to suppress DMD symptoms.	Continuous administration.	Only useful for punctual mutation's patients (10-30%).	Continuous administration. Target design patient-specific	Difficulties to arrive to all the target locations using an *in vivo* approach.	Only in research.

MATERIALS AND METHODS

In this project, the most recent publications related to DMD treatment and its therapeutic approaches, either researches or clinical trials were studied.

Data has been obtained using the searching engine *Pubmed*. The search was based in key words such as *Duchenne, gene therapy* and *iPSC.* These articles were read and summarized.

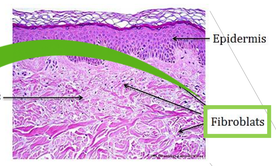
Afterwards, different strategies and methods were compared based on risks and benefits and the best one was selected and explained.

THERAPEUTIC APPROACH DESIGN

Autologous *ex vivo* cell therapy

- Patient cells = donor cells → ✖ immune response
- DMD patients have ↓ myoblasts or satellite cells
- Somatic cells → iPSCs

Skin biopsy



- Same embryonic origin (mesenchyma)
- Easy to obtain, culture and expansion
- Simple nutritive request and culture viability

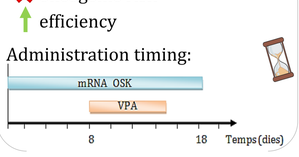
Fibroblasts[2]

Oct4 mRNA
Klf4 mRNA
Sox2 mRNA
VPA

reprogramming[3,4]

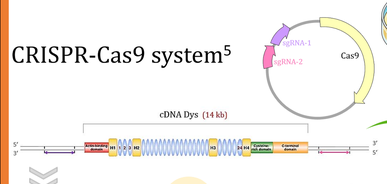
✖ oncogenic risk
↑ efficiency

Administration timing:

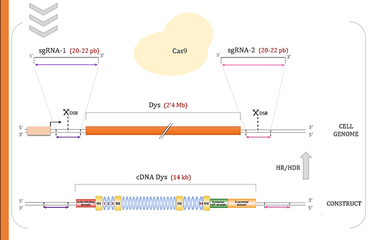


iPSC

CRISPR-Cas9 system[5]



ex vivo gene therapy



⚠ clones selection
✖ off-target[5]

iPSC^{Dys}

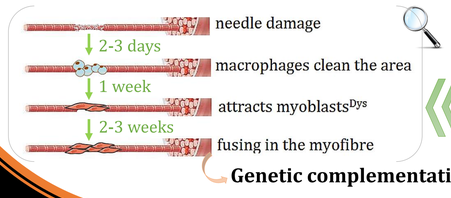
differentiation[2,4]

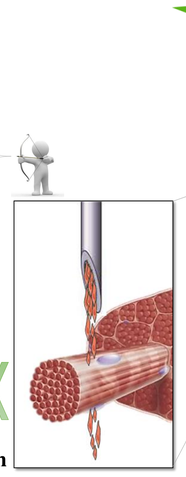
MyoD mRNA (3 days)

Myoblast^{Dys}

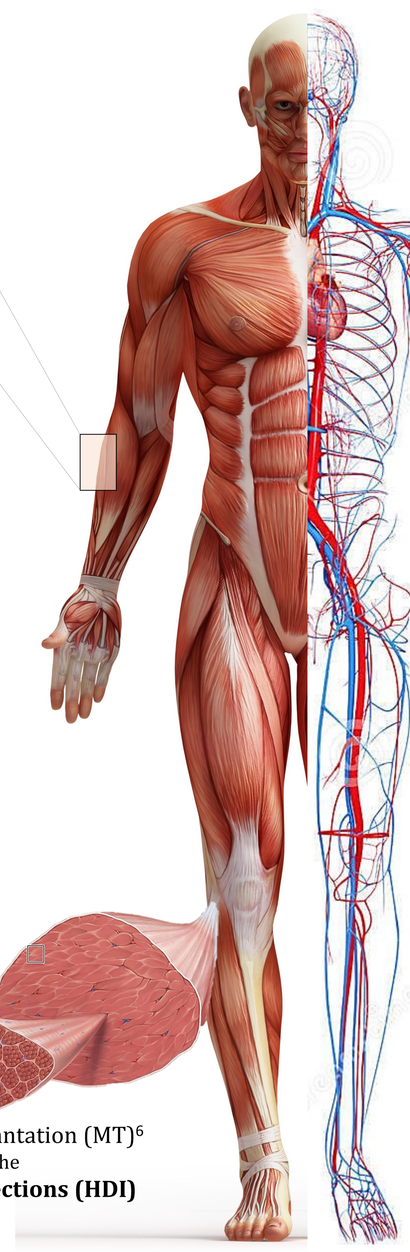
Target locomotors muscles:

- Upper limb: deltoid, biceps, triceps, brachioradialis
- Lower limb: quadriceps, sartorius, tibialis anterior, biceps femoral, semimembranosus, semitendinosus, gastrocnemius





Myoblasts transplantation (MT)[6] due to the **high-density injections (HDI)**



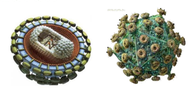
Intravascular administration
Medium vein

⚠ single administration

In vivo gene therapy

- DMD patients' cause of death: **cardiorespiratory failure**
- Target tissues: **myocardium + respiratory muscles** (differentiated cells)
- Minimal levels of functional dystrophin expression to ameliorate symptoms of DMD: **>40-50%**

VECTOR[1,7]



Retrovirus and Lentivirus	Adenovirus	Adeno-associated viral vectors (AAV)	Non-viral vectors
Infected replicative cells (lentivirus also quiescent) Integrative vector Insertional mutagenesis	Infected replicative and quiescent cells Non-integrative vector ↑ immunogenic	Infected replicative and quiescent cells Non-integrative vector ↓ immunogenic	↓ efficiency

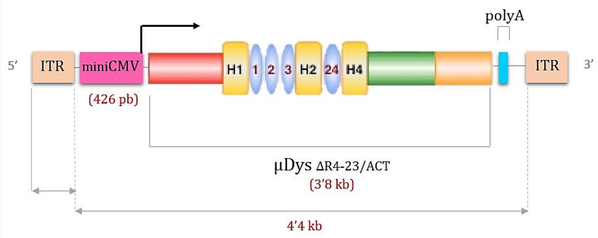
Target tissues' tropism of AAVs serotypes[7]:

- **AAV2/1:** — myocardium ↑↑ skeletal muscle (via IM)
- **AAV2/8:** ↑ myocardium ↑↑ skeletal muscle (via IV)
- **AAV2/9:** ↑↑ myocardium ↑ skeletal muscle (via IV)

⚠ encapsidation capacity: **4'7 kb** (4'4 kb + 2x ITR) [1]

✖ cDNA dystrophin (14 kb)

CONSTRUCT DESIGN

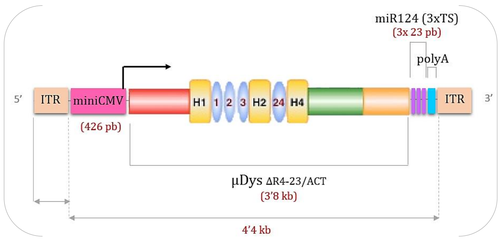


- **µDys**: synthetic abbreviate functional dystrophin.[1]
- **miniCMV**: ubiquous and powerful promoter. It is been silenciate in the liver of large animals.

It would be better to use tissue specific promoters, such as MCK for skeletal muscle or MLC2v for myocardium, but they are too big for an AAV size.

⚠ AAV2/9 also has a good tropism to liver and CNS, due to it can cross BHE[7].

Even though studies do not show any toxicity or side effect in these localizations, if in following studies it detects someone, an alternative construct could be used.



Alternative construct

mirR-T: targeting sequence of a tissue specific microRNA.

microRNAs silence the expression of mRNAs. If the construct has got the targeting sequence of a CNS specific miR-T, such as TS miR124, the transgen will not express in this tissue.

IMMUNOLOGICAL REMARKS

- If the patient has anti-AAV antibodies before treatment, he cannot be accepted for the therapy because the treatment is not going to be effective.
- Immunosuppressants have to be administrated until patient eliminates viral capsids.[7]
- Depending on the genetic alteration, the immune systems of patients could react against new dystrophin (*ex vivo* cell therapy) or synthetic abbreviation dystrofin (*in vivo* gene therapy). For these reason, some patients have to take immunosuppressant in the lifelong.[6]

REFERENCES

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3. Maherali *et al.* Cell Stem Cell. 2008;3:595-605
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CONCLUSIONS

- Nowadays, it does not exist a clinical strategy which completely restore healthy phenotype in Duchenne patients.
- Nevertheless, an optimal therapeutic approach has been determinated. This one ameliorate locomotor function and prolong life expectancy, like clinical characteristics of Becker muscular dystrophy patients.
- More studies in mice (*mdx*) and canine (*cxmd*) models are needed to further develop this therapy.