Use of CRISPR/Cas9 in viral gene therapy to treat Duchenne muscular dystrophy

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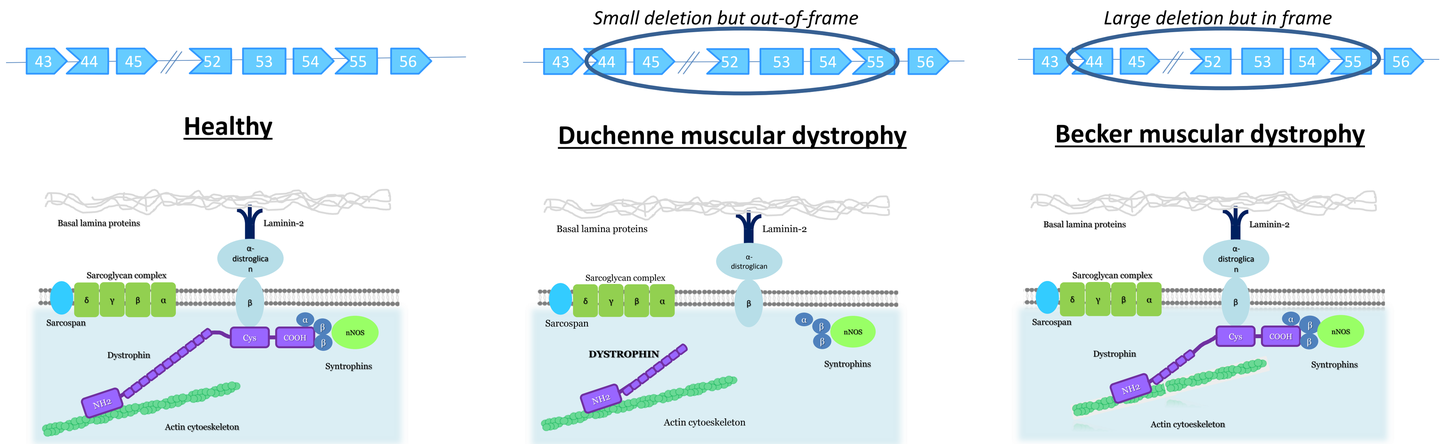
Introduction to Duchenne muscular dystrophy and CRISPR/Cas9 technology

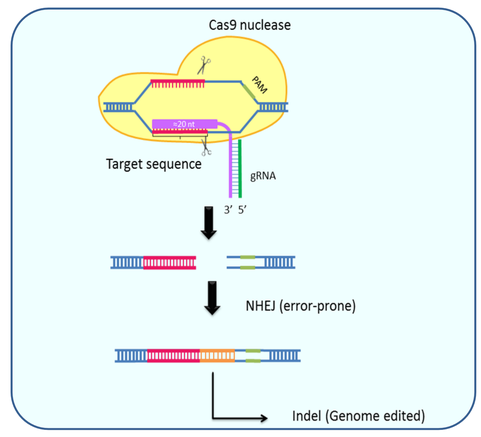
Which is the cause of DMD?

DMD is a is an X-linked recessive disorder caused by mutations in dystrophin gene, that provokes a non-functional protein. When this mutations appear protein loses its capacity to bind internal cytoskeleton to extracellular matrix in muscular tissue. DMD patients suffer from muscular degeneration, that over time becomes life-threatening. In contrast, BMD patients develop milder and later symptoms as the mutations lead to a truncated but partially functional dystrophin

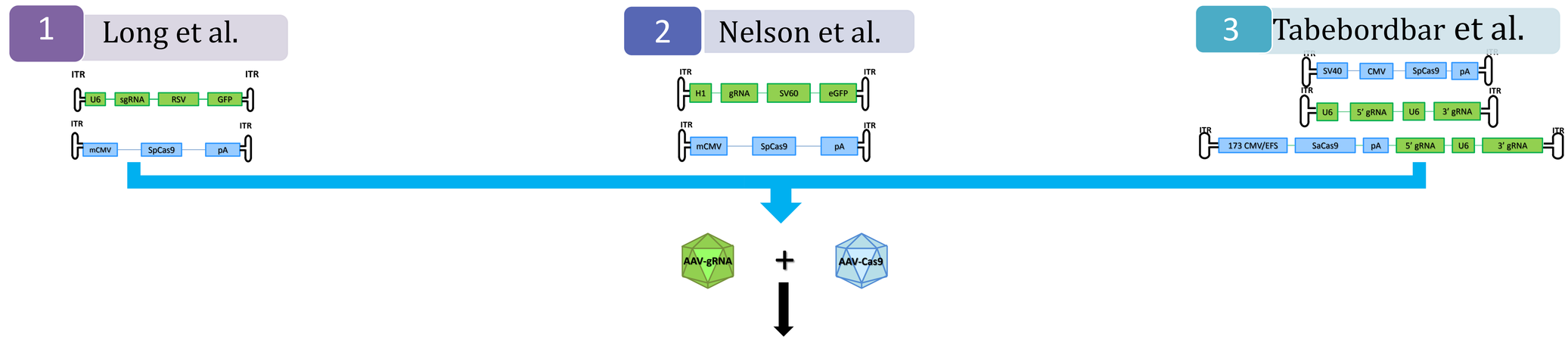


How does CRISPR/Cas9 technology work?

CRISPR/Cas9 is a genome editing tool that uses a guide RNA (gRNA) and a specific nuclease (Cas9) to cleave a targeted sequence. The result will be a double-strand break in the DNA sequence, which the cell will try to repair by either HDR (homology-directed repair) or NHEJ (non-homologous end-joining), performing the genome edition.



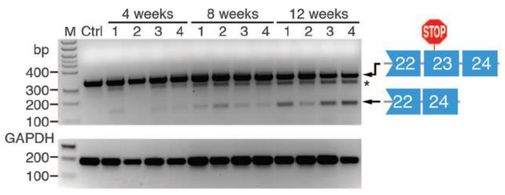
3 Approaches for gene therapy



RESULTS

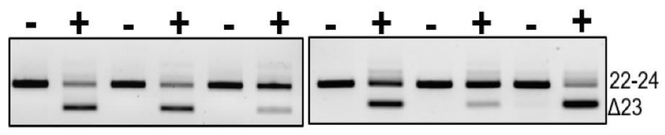
EXON EXCISED

Long et al study



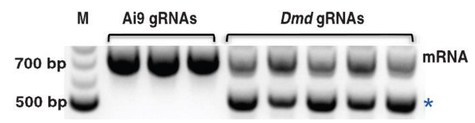
RT-PCR from four mice after AAV-retraorbital injection shows the presence of an excised band in time, demonstrating that the CRISPR/Cas9 method had excised exon 23

Nelson et al study



RT-PCR shows an only band in non-treated animals, in contrast to AAV-CRISPR/Cas9 treated mice that show two bands, the lower corresponding to the missing defective exon 23.

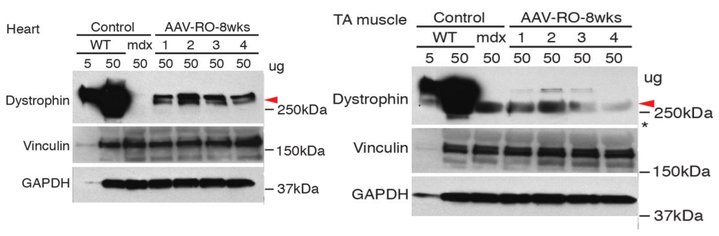
Tabebordbar et al study



RT-PCR shows again a non excised band of 700 pb in all mice and in animals treated with AAV-*Dmd* an additional 500 bp band corresponding to the excised exon.

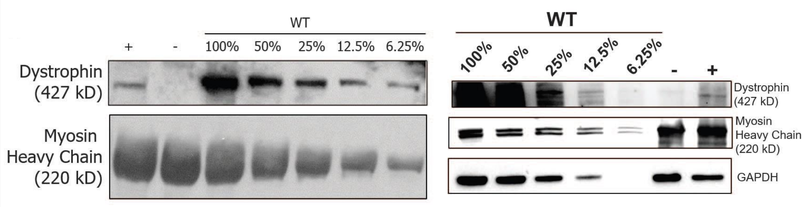
DYSTROPHIN RESTORED

Long et al study



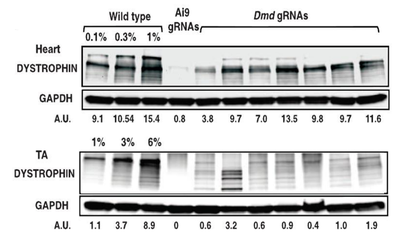
A Western blot was performed to see the dystrophin presence, in this case with 4 mice treated by retraorbital injection at 8 weeks, comparing skeletal TA and heart muscles.

Nelson et al study



The Western blot analysis from Nelson et al. confirmed the presence of dystrophin after the treatment as shown in the positive controls (+). Moreover, the intensity quantification confirms a presence of at least 6% of dystrophin in TA muscle and 12% in heart muscle.

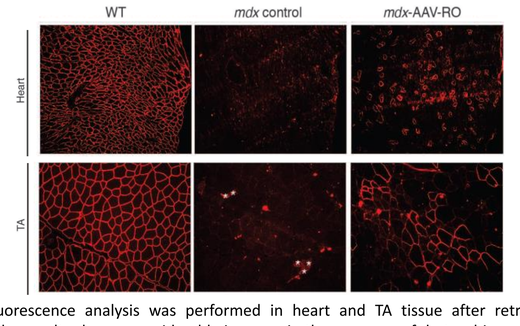
Tabebordbar et al study



The Western blot assay confirmed the presence of dystrophin after the treatment as shown in 7 mice treated with Dmd gRNAs. The intensity quantification confirms the presence of dystrophin in both heart and TA muscle, compared with the WT and the negative control.

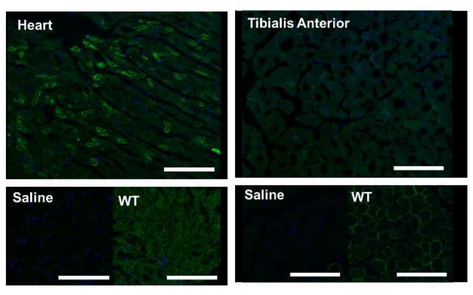
DYSTROPHIN PRESENCE

Long et al study



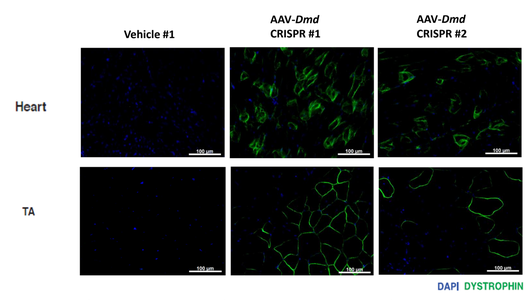
An immunofluorescence analysis was performed in heart and TA tissue after retraorbital injection and the results show a considerable increase in the presence of dystrophin comparing with mdx phenotype

Nelson et al study



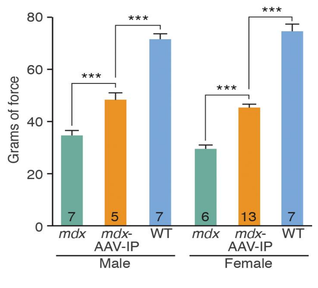
The immunofluorescence comparison between WT, saline (Mdx non-treated) and treated mice show that after AAV-CRISPR intraperitoneal injection, heart and TA have significantly recovered dystrophin.

Tabebordbar et al study

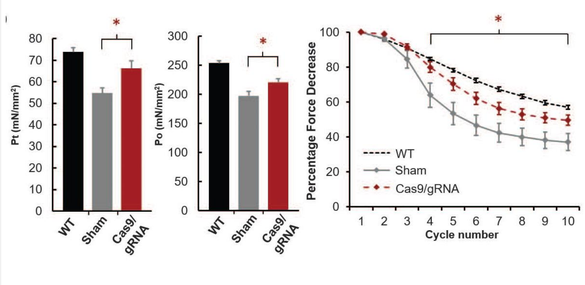


The immunofluorescence assay from two treated mice show an increase in the presence of dystrophin in both heart and skeletal muscle cells in comparison to non-treated (vehicle).

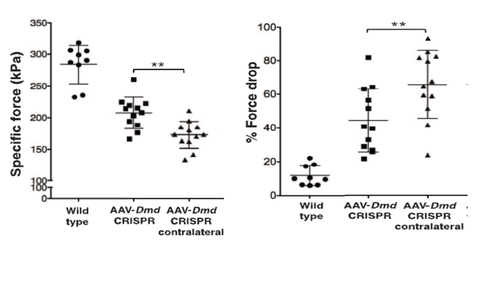
MUSCLE FORCE



Long et al. performed a *grip strength test* in male and female mice 4 weeks after intraperitoneal injection and the results show a significant improvement of the muscle force.



In situ muscle force test (Aurora) shows a significant improvement in both increase in muscle force and a decrease in muscle force drop in treated mice as seen in the graphics.



In situ muscle force test (Aurora) from Tabebordbar shows a very slight improvement of treated mice in muscle force and a decrease in muscle force drop.

Conclusions

- This novel approach is optimistic as results have shown a significant recovery of dystrophin levels and muscle force.
- The fact of the use of CRISPR/Cas9 enables this therapy to be easier and faster than other therapies used in DMD.
- Off-target effects are a concern and need to be accurately analysed in order to guarantee more effective and safer results in this therapy.
- Another concern is vector delivery and production, as it is now one of the main burden in gene therapy, so there should be more study in vector optimization and production.
- Bioethical implications need to be considered when reaching into Clinical Phase, as the patients involved are being children and this is a great deal.

References

1. "*Postnatal genome editing partially restores dystrophin expression in a mouse model of muscular dystrophy*". **Long** C, Amoasii L, Mireault AA et al. Science. 2016 Jan 22;351(6271):400-3.
2. *"In vivo genome editing improves muscle function in a mouse model of Duchenne muscular dystrophy*". **Nelson** CE, Ousterout DG et al. Science. 2016 Jan 22;351(6271):403-7.
3. *"In vivo gene editing in dystrophic mouse muscle and muscle stem cells".* **Tabebordbar** M, Zhu K, Cheng JK et al. Science. 2016 Jan 22;351(6271):407-11.
4. *Development and Applications of CRISPR-Cas9 for genome engineering"*. **Patrick D. Hsu**, Eric S. Lander, Feng Zhang. Cell, Volume 157, Issue 6 5 June 2014.