

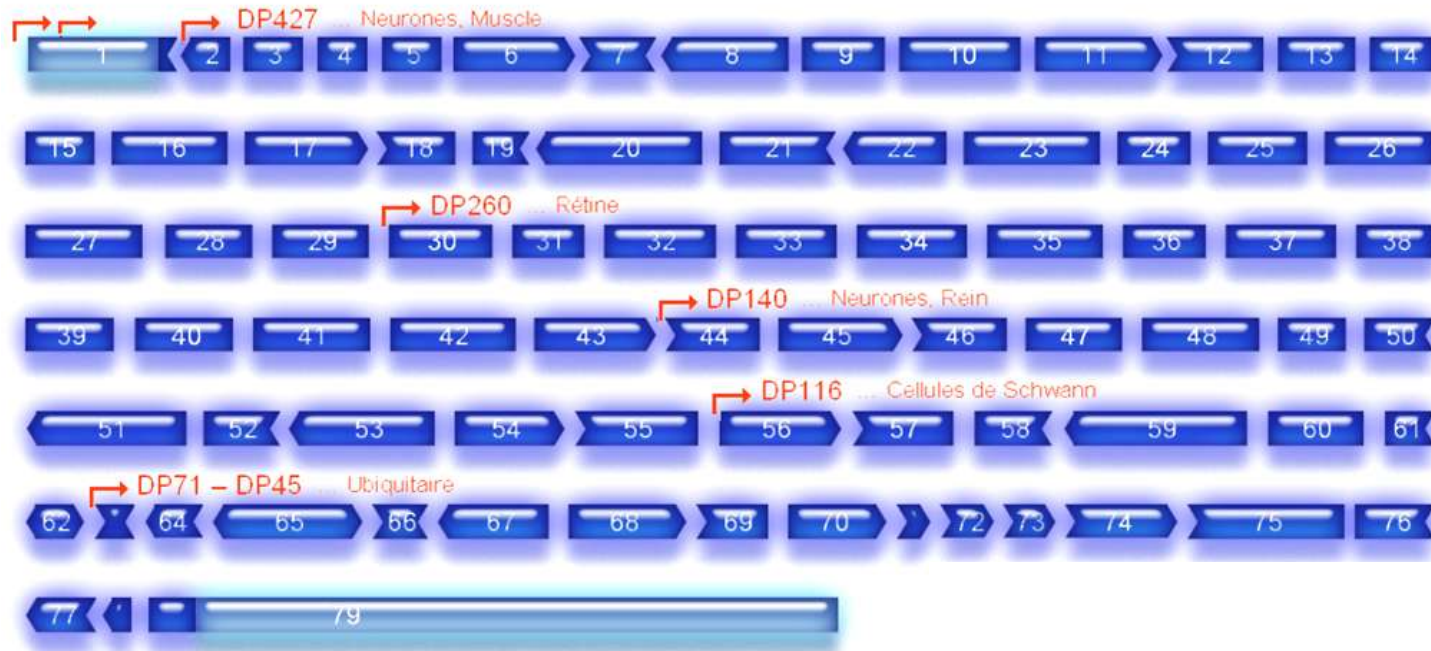
Multi Exon-skipping Approach for Duchenne Muscular Dystrophy Therapy

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DMD gene and dystrophin



⇒ DMD gene is composed of 79 segments called « exons »

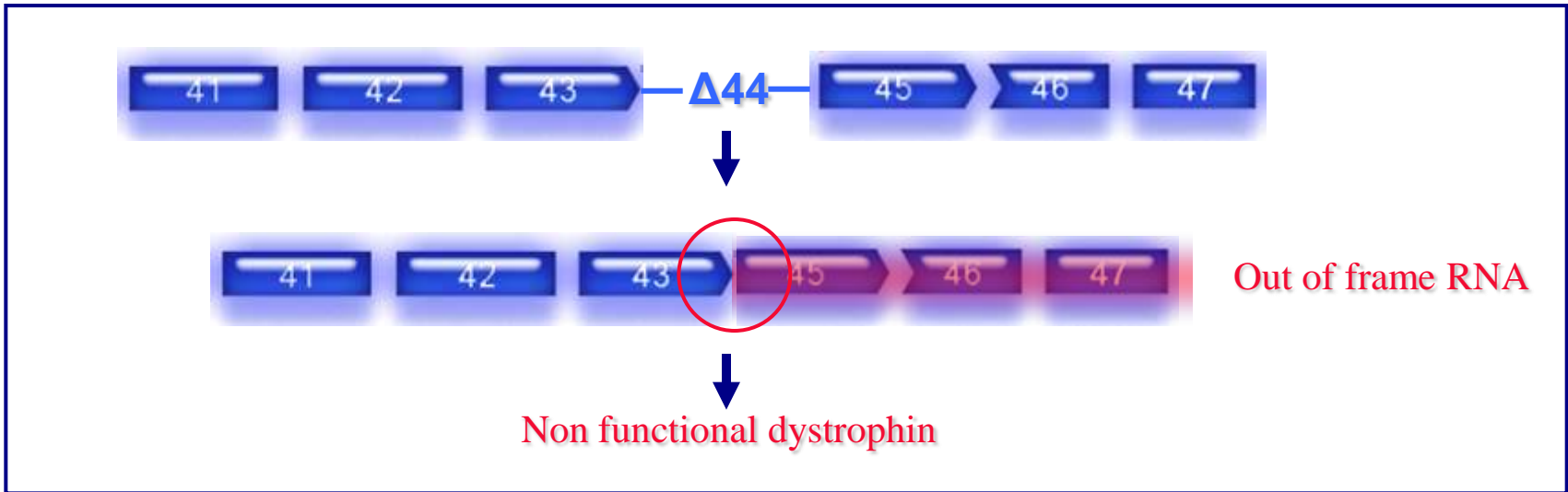
⇒ These 79 exons are joined together and form a message

⇒ The message gives rise to the dystrophin protein

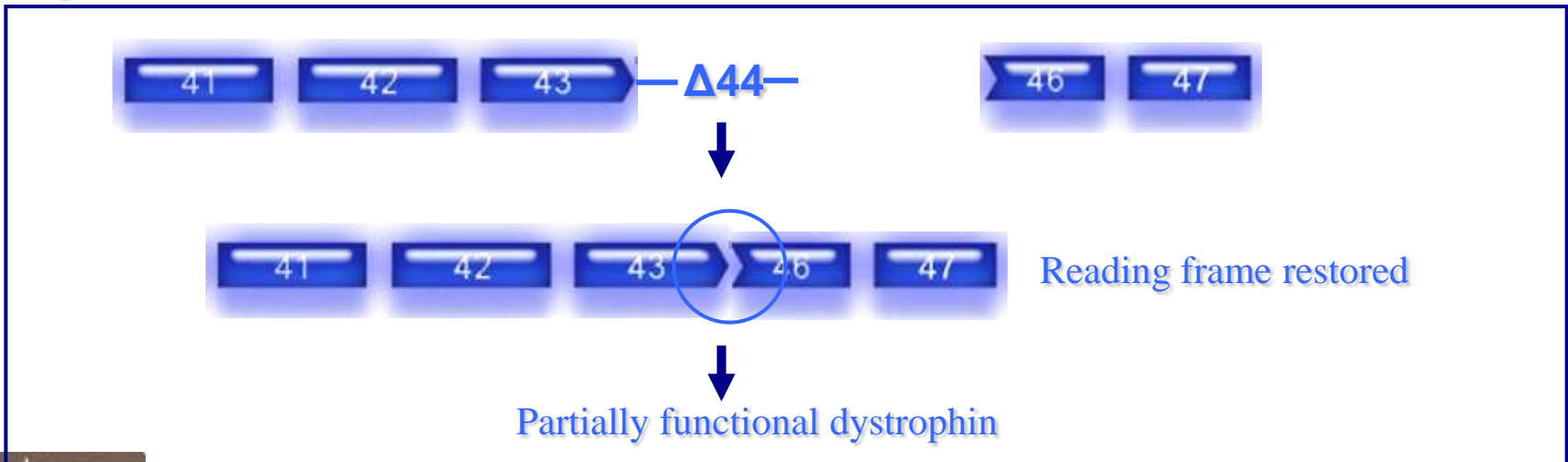


Deletions in the DMD gene

Deletion of exon 44



Reading frame restoration



Strategies to achieve exon-skipping

• Antisense Oligonucleotides

Clinical trials have showed encouraging results

- Local and systemic administrations showed promising results with 2 different chemistries
- Challenges remain:
 - ⇒ Periodic administration required – long term effects need to be evaluated
 - ⇒ Improving delivery, especially to cardiac muscle
 - ⇒ Develop Antisense oligonucleotide for other exons

• Antisense sequences vectorized into viral vectors

AAV vectors can deliver gene very efficiently to muscle

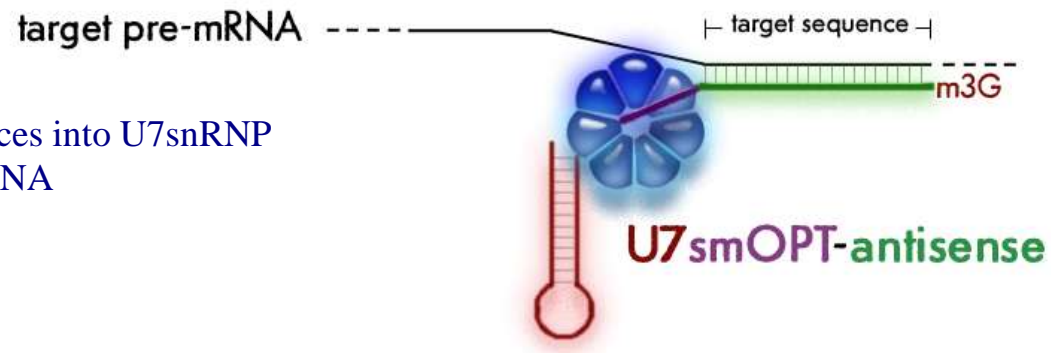
- Long term effect
- Efficient systemic delivery
- Can deliver multiple « antisense oligonucleotides »



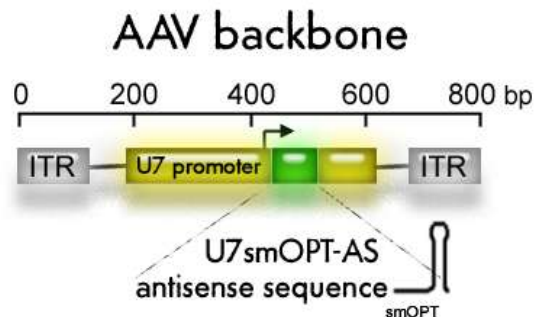
« Antisense oligonucleotides » are carried by U7snRNA gene

⇒ U7snRNA gene as a carrier :

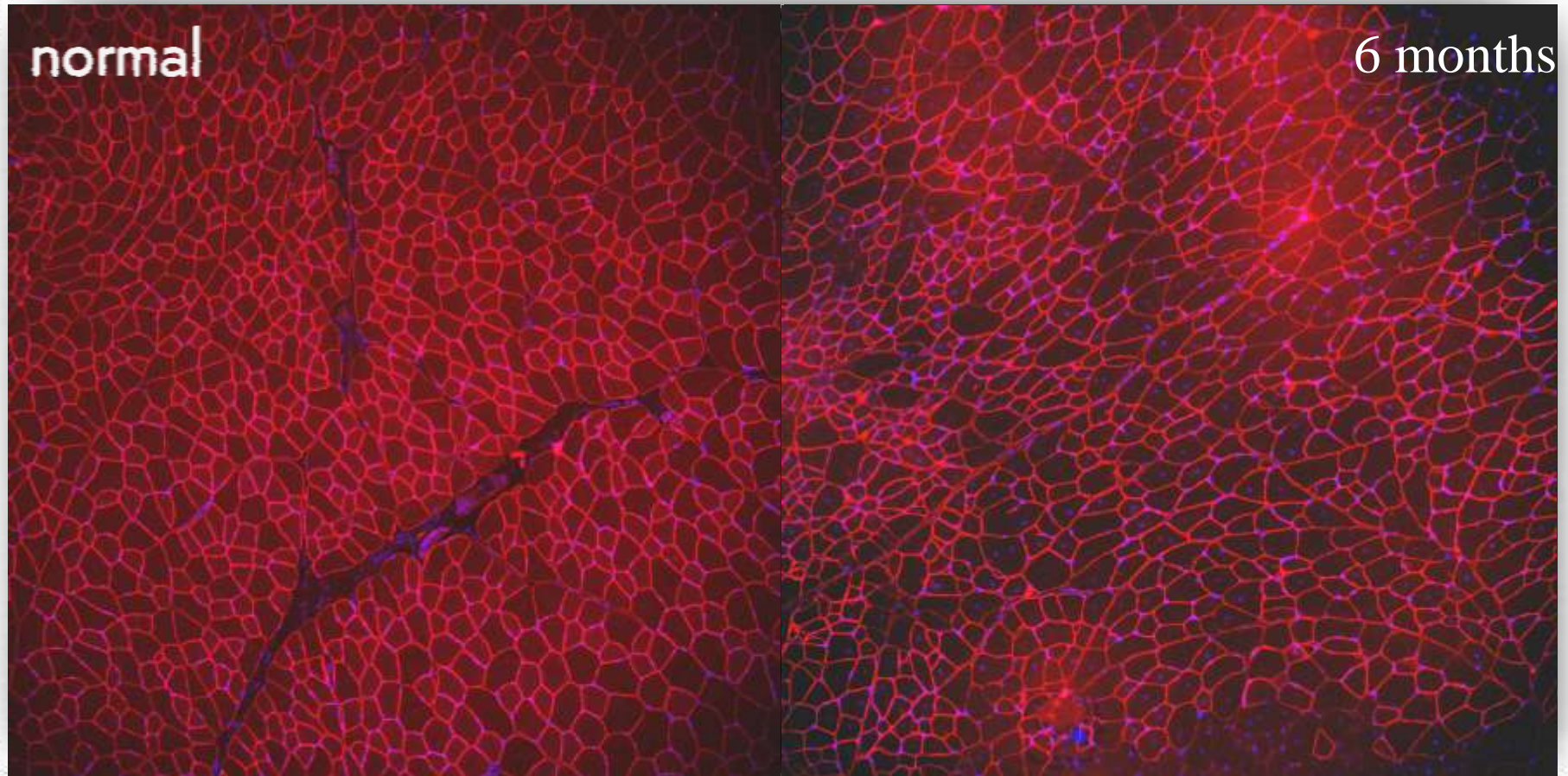
- Stabilisation of antisense sequences into U7snRNP
- Colocalisation with target pre-mRNA



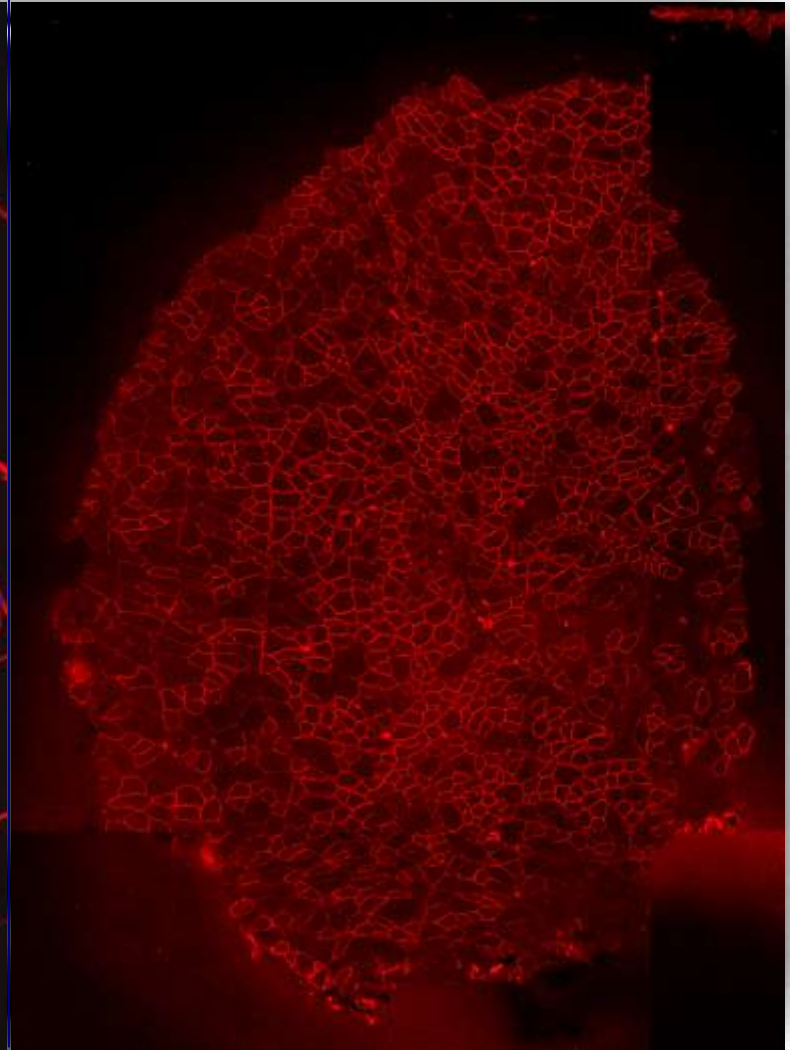
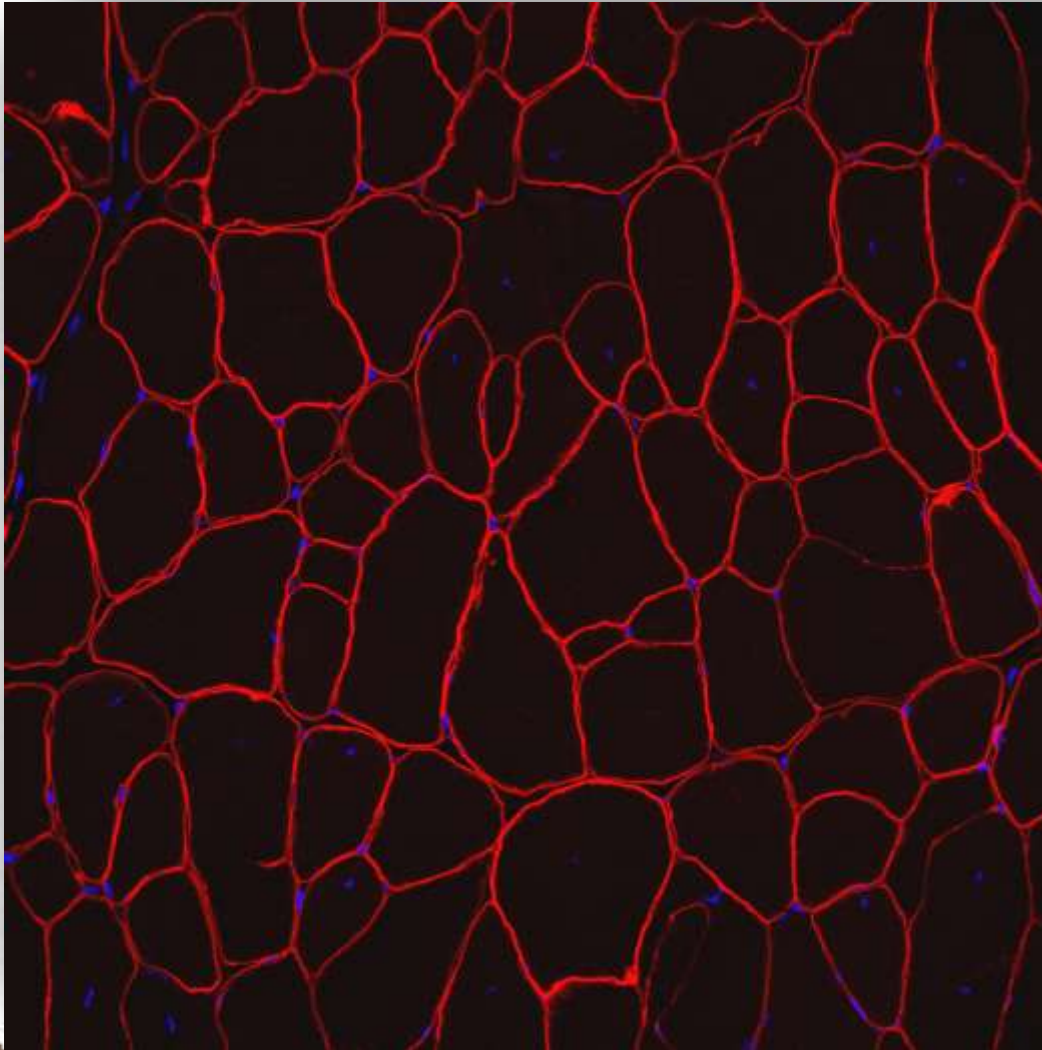
⇒ Production of AAV vectors encoding modified U7snRNA



Dystrophin rescue after 1 single injection in mdx mouse



One year after a single injection

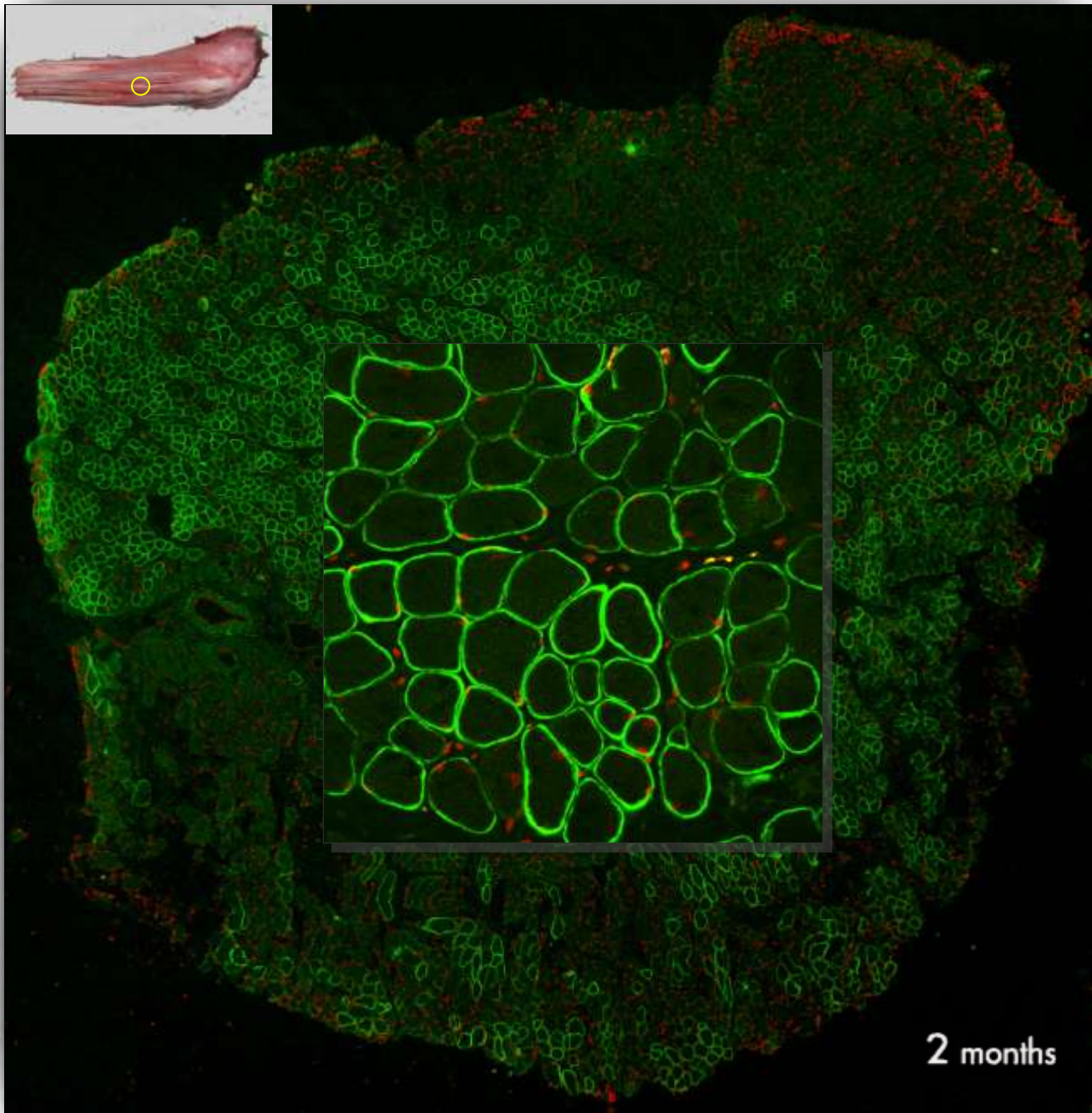


Widespread rescue of dystrophin in GRMD forelimb

- Evaluation of Locoregional delivery of AAV-U7snRNA



Collaboration with Institute of Myology and Veterinary School of Maison Alfort, France



2 months

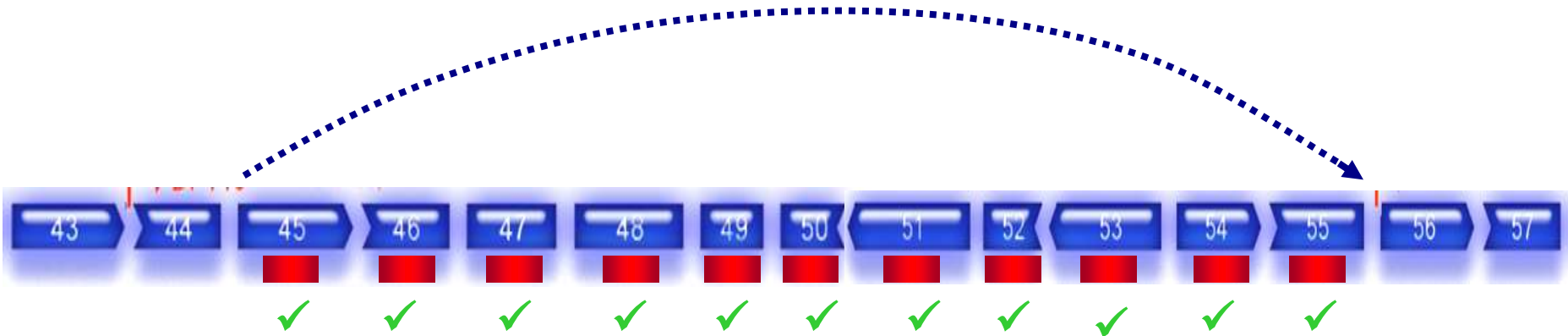
Applicability of exon-skipping and potential of AAV delivery

- Exon-skipping would be applicable to 83% of DMD patients
- Treatment of DMD using exon-skipping will require different plasters adapted to the patients mutation
 - Clinical trials have investigated exon 51 skipping, which would be applicable to 13% of patients
 - ↳ Other plasters have to be developed for the other exons
 - Regulatory agencies consider each new « molecular plaster » as a new drug, that will have to go through the expensive and lengthy clinical trial stages
- ↳ **Development of a single viral vector which would deliver multiple plasters could be applicable to 63% of DMD patients**

Multi exon-skipping approach

The multiple skipping of exons 45 to 55 would be applicable to 63% of DMD patients

⇒ Targeting all 11 exons from 45 to 55 :



⇒ Design efficient U7 construct for each exon (mono-skipping)

- *In vitro* evaluation in muscle cells from DMD patients
- *In vivo* in mouse model of DMD

Part I : Design efficient U7snRNA for each exon

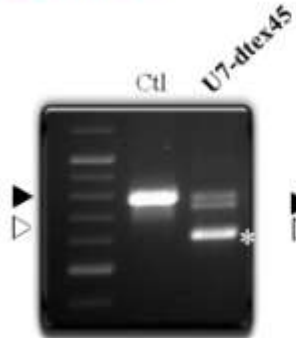
⇒ Engineer various U7 constructs for each exon between 45 and 55

- for 11 exons : total 41 constructions

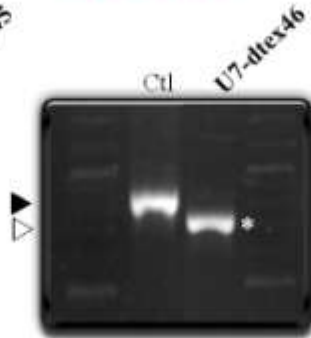
⇒ *In vitro* evaluation in human myoblasts

- lentiviral production, myoblasts transduction and further RNA analysis

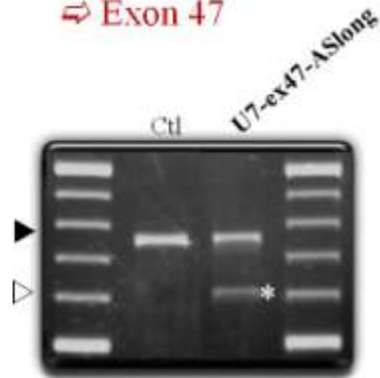
⇒ Exon 45



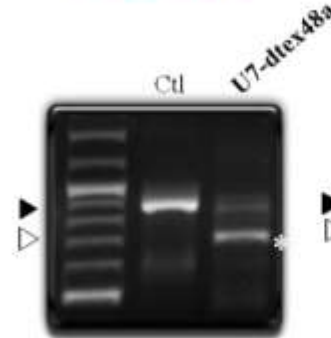
⇒ Exon 46



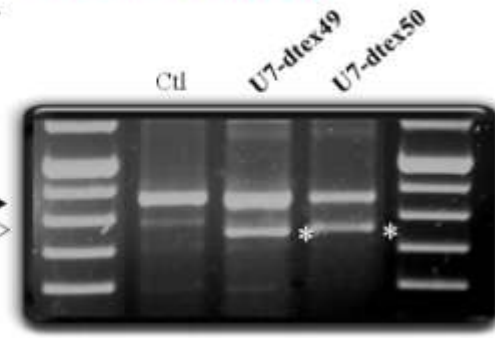
⇒ Exon 47



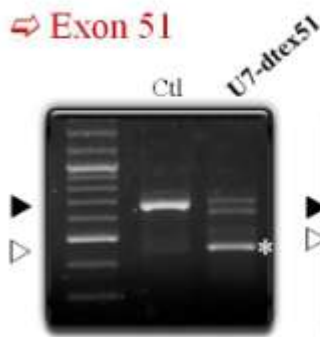
⇒ Exon 48



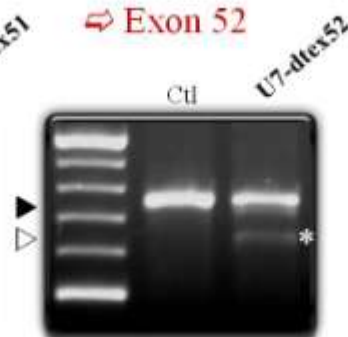
⇒ Exons 49 and 50



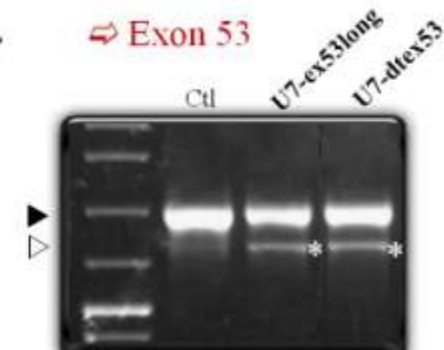
⇒ Exon 51



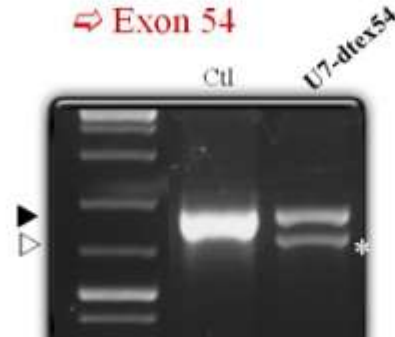
⇒ Exon 52



⇒ Exon 53



⇒ Exon 54

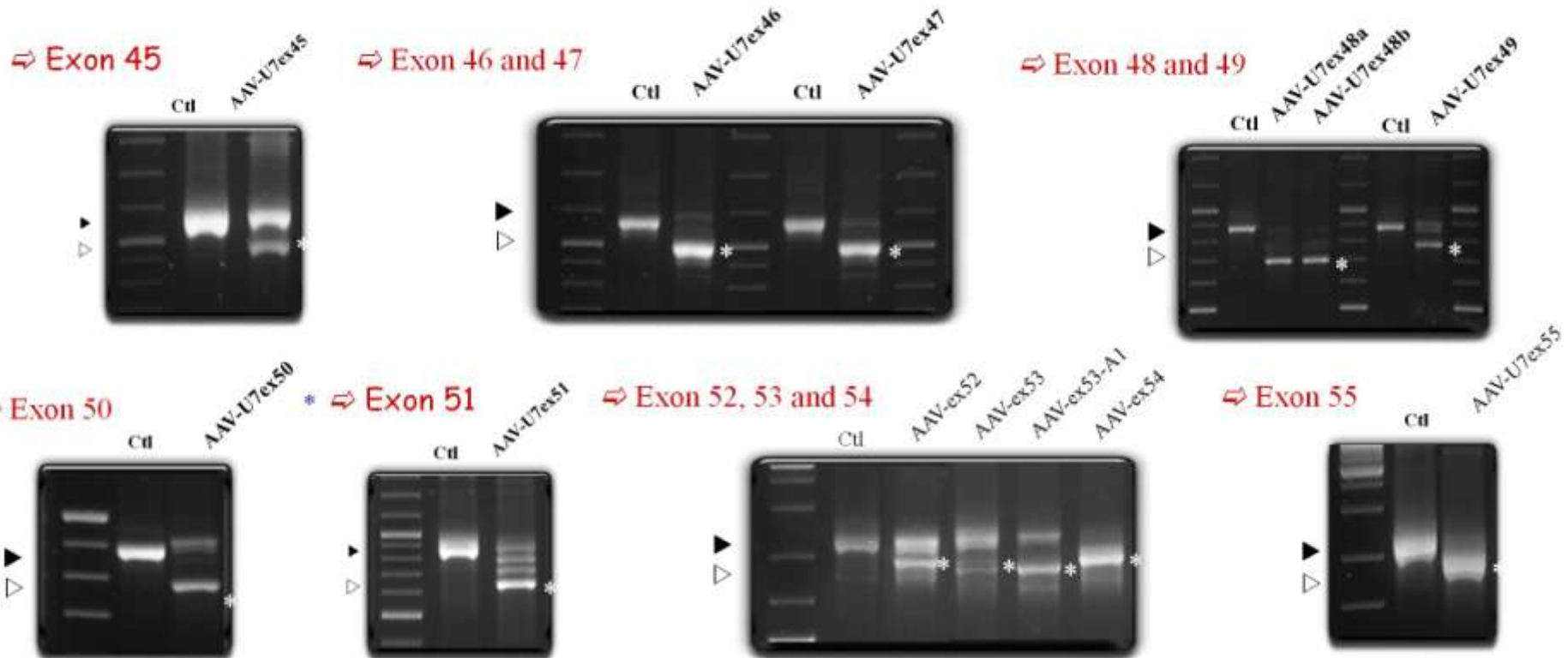


⇒ Exon 55



In vivo evaluation of U7 constructs targeting human exons

- ⇒ Intramuscular injection of AAV-U7 vectors in hDMD mice
 - selection of best constructs based on *in vitro* data (1 or 2 per exon)
 - AAV vector production
- IM injection in hDMD mice and skipping analysis 4 weeks post-injection

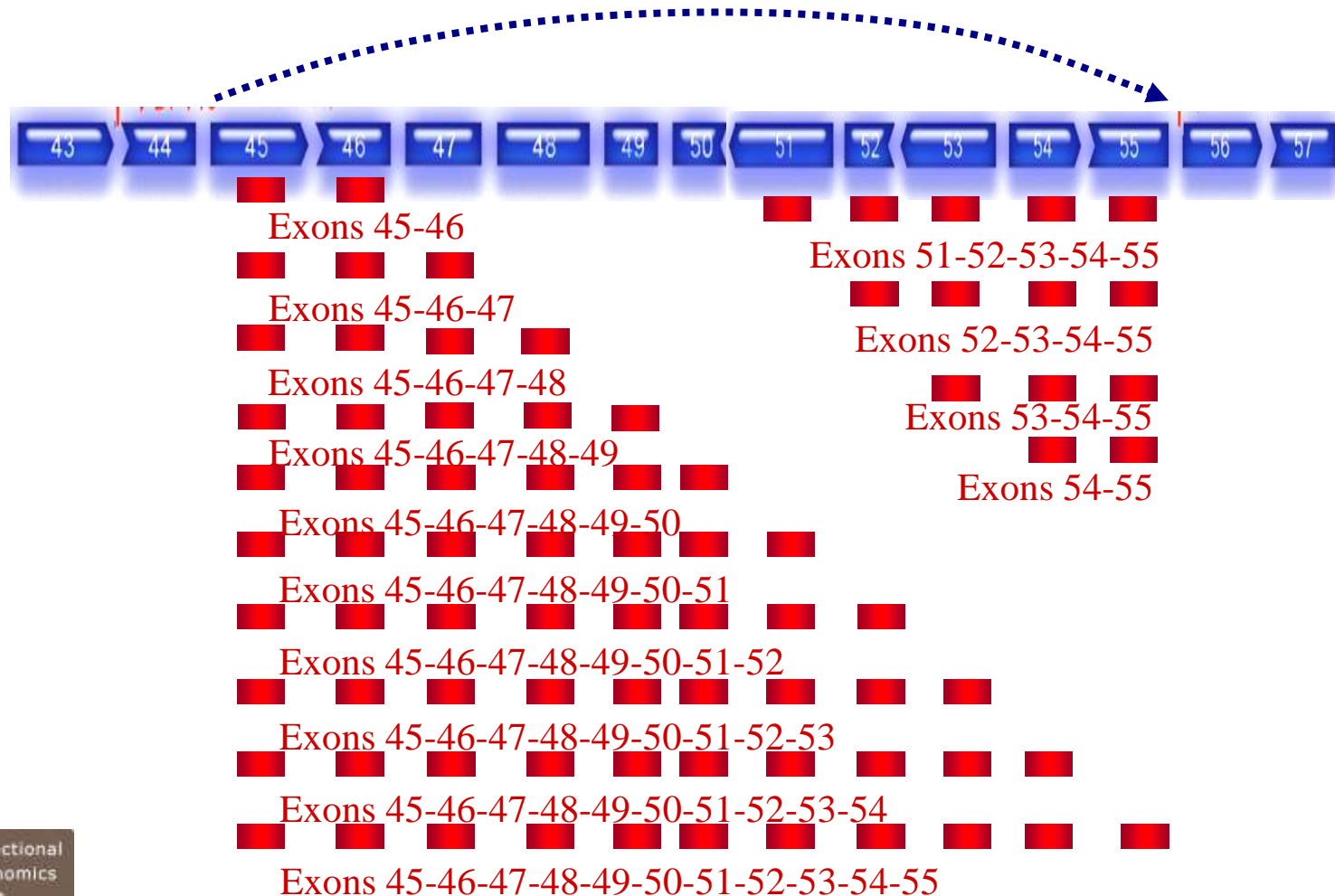


AAV mediated Multi exon-skipping



⇒ Engineer a single AAV vector to deliver multiple plasters

- Design all single plasters, combine in a single vector, produce vector for *in vitro* test in cells from DMD patients, evaluate dystrophin restoration, test *in vivo* in mouse model

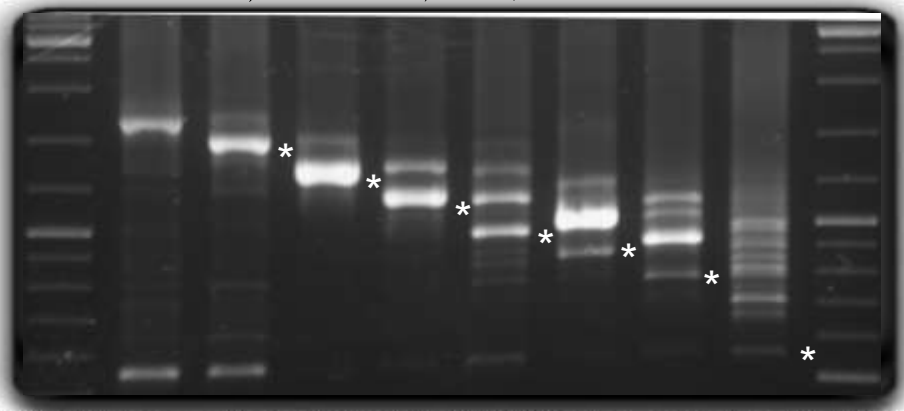


Promising results on Multi exon-skipping

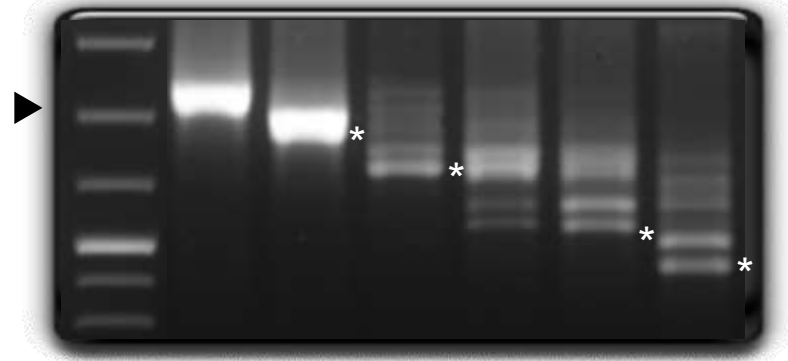
⇒ Evaluation of these multiple combinations directly into muscles from mouse model



Ctl AAV-ex46 AAV-ex45-46 AAV-ex45-46-47 AAV-ex45-46-47-48 AAV-ex45-46-47-48-49 AAV-ex45-46-47-48-49-50 AAV-ex45-46-47-48-49-50-51



Ctl AAV-ex54 AAV-ex54-55 AAV-ex53-54-55 AAV-ex52-53-54-55



Conclusions

- AAV-U7snRNA can induce efficient Exon-skipping and Long term restoration of dystrophin
- Efficient systemic delivery have been shown in mouse and dog models
 - Challenges remain regarding the optimal dose and immune response
 - ⇒ Pre-clinical studies on GRMD dogs are currently performed by Genethon, The Institute of myology in collaboration with the veterinary school of Nantes
- AAV vectors can deliver multiple U7snRNA and induce multi Exon skipping
 - ⇒ Optimisation of multi Vectors on Going

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