Use of Aptamers to deliver therapeutic genetic sequences in muscle

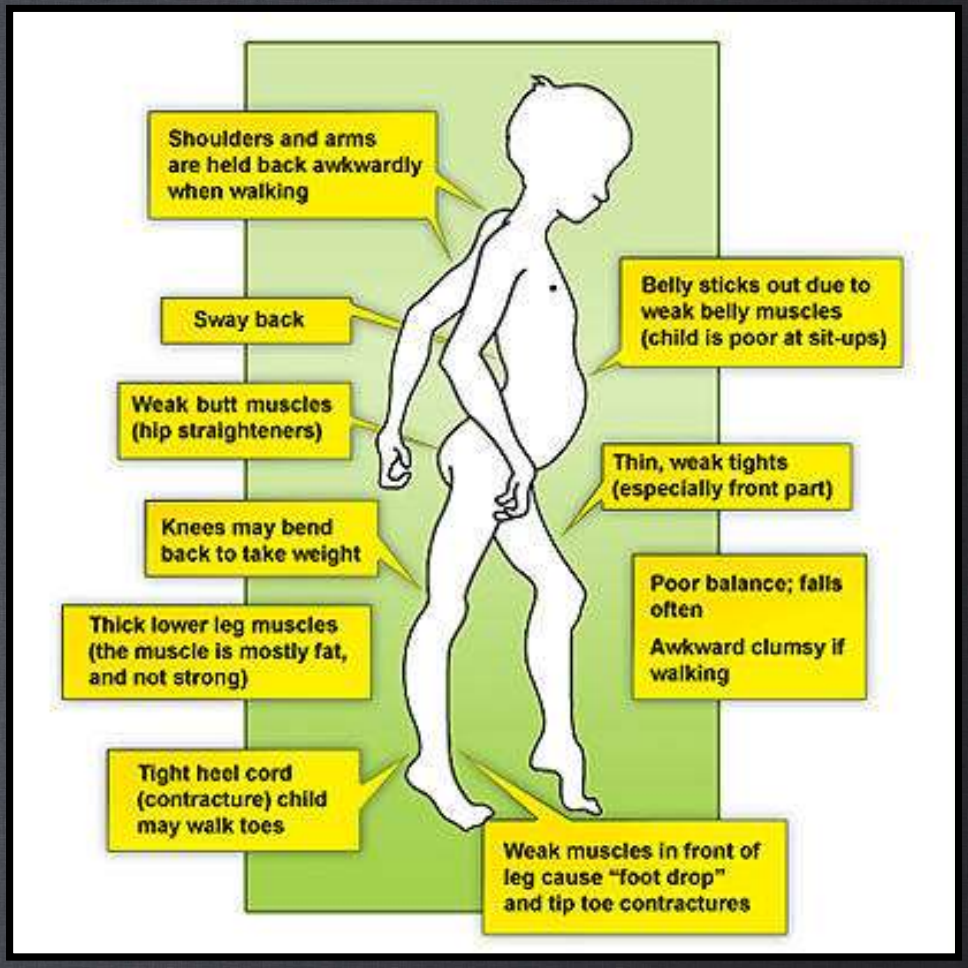
LEONIDAS A. PHYLACTOU

THE CYPRUS INSTITUTE OF NEUROLOGY & GENETICS

CYPRUS SCHOOL of molecular medicine
A SCHOOL OF THE CYPRUS INSTITUTE OF NEUROLOGY & GENETICS

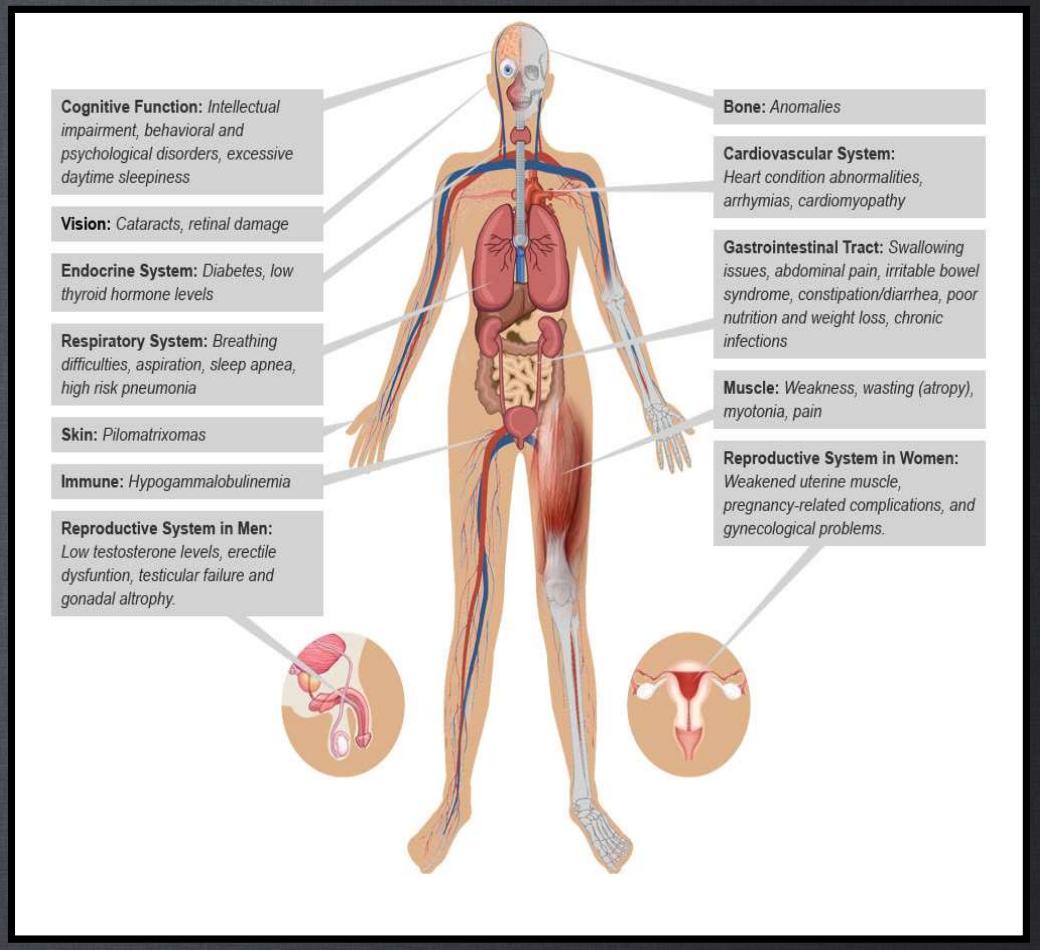
Muscular Dystrophy

- Group of muscle diseases
- Inherited
- Muscle weakness and wasting
- Duchenne Muscular Dystrophy, Becker Muscular Dystrophy, Myotonic Dystrophy



MYOTONIC Dystrophy

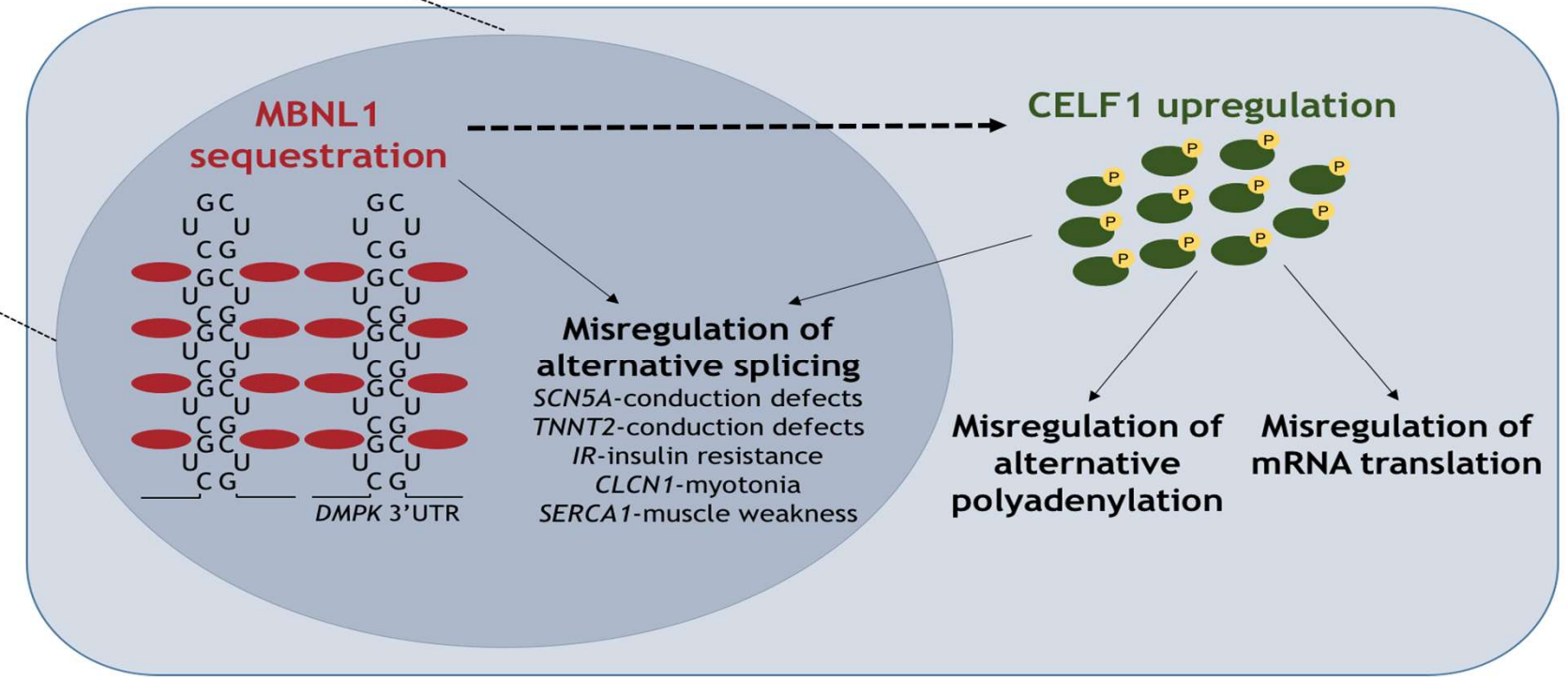
- Autosomal dominant
- Most common neuromuscular disease in adults
- Muscle weakness and wasting
- Other symptoms may include cataracts, intellectual disability, and heart conduction problems
- Type 1 (DM1) and type 2 (DM2)
- Anticipation



Thornton, C. A. (2014). "Myotonic dystrophy." Neurologic clinics 32(3): 705-719, viii.

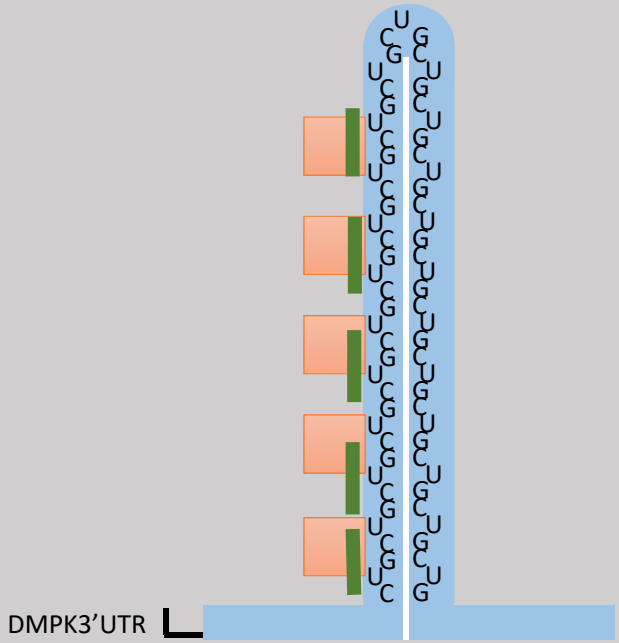
DM1 Pathogenesis

Nuclear retention of CUG^{exp}-MBNL1 foci



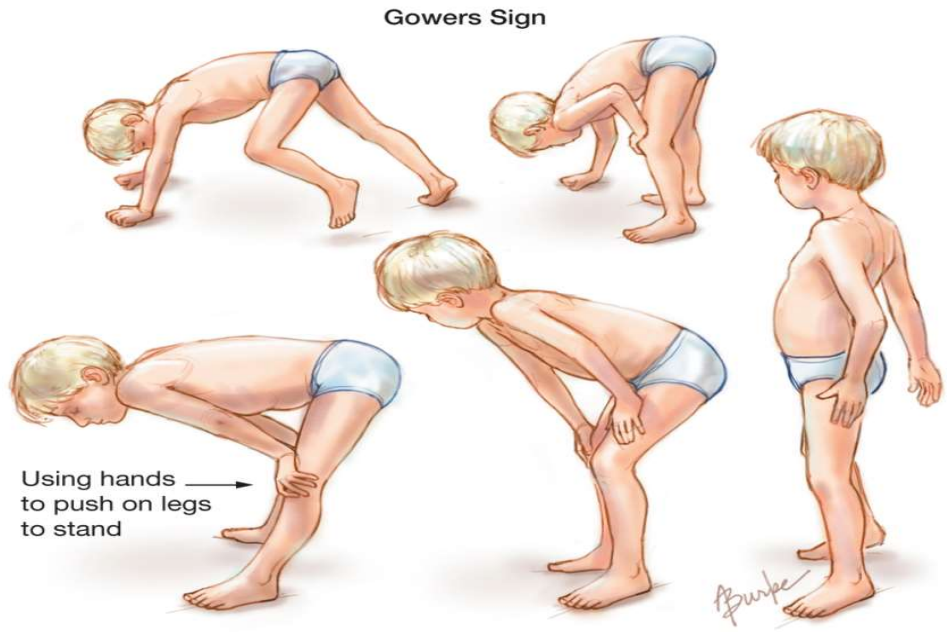
Thornton, C. A. (2014). "Myotonic dystrophy." Neurologic clinics 32(3): 705-719, viii.

ANTISENSE OLIGONUCLEOTIDES AGAINST MYOTONIC DYSTROPHY



Duchenne muscular Dystrophy

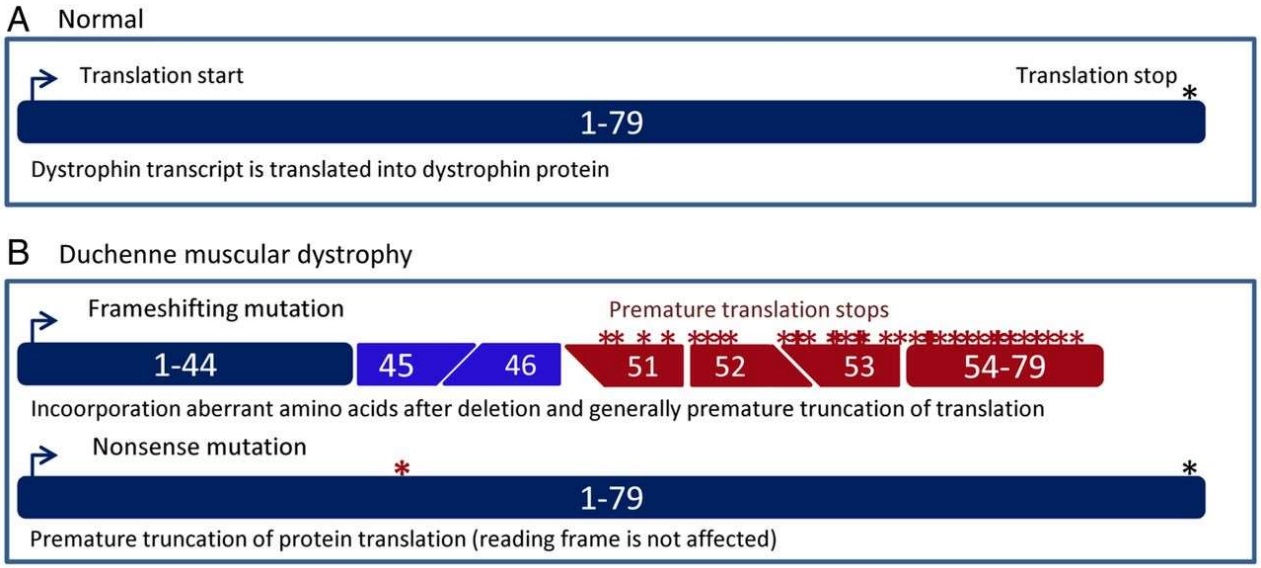
- X-linked
- Most common form of muscular dystrophy
- 1 in 3,500 newborn boys
- Progressive muscle weakness and cardiomyopathy
- Ultimately die from cardiac or respiratory complications before their third decade of life



Chamberlain, J. R. and J. S. Chamberlain (2017). Molecular Therapy 25(5): 1125-1131

DMD gene

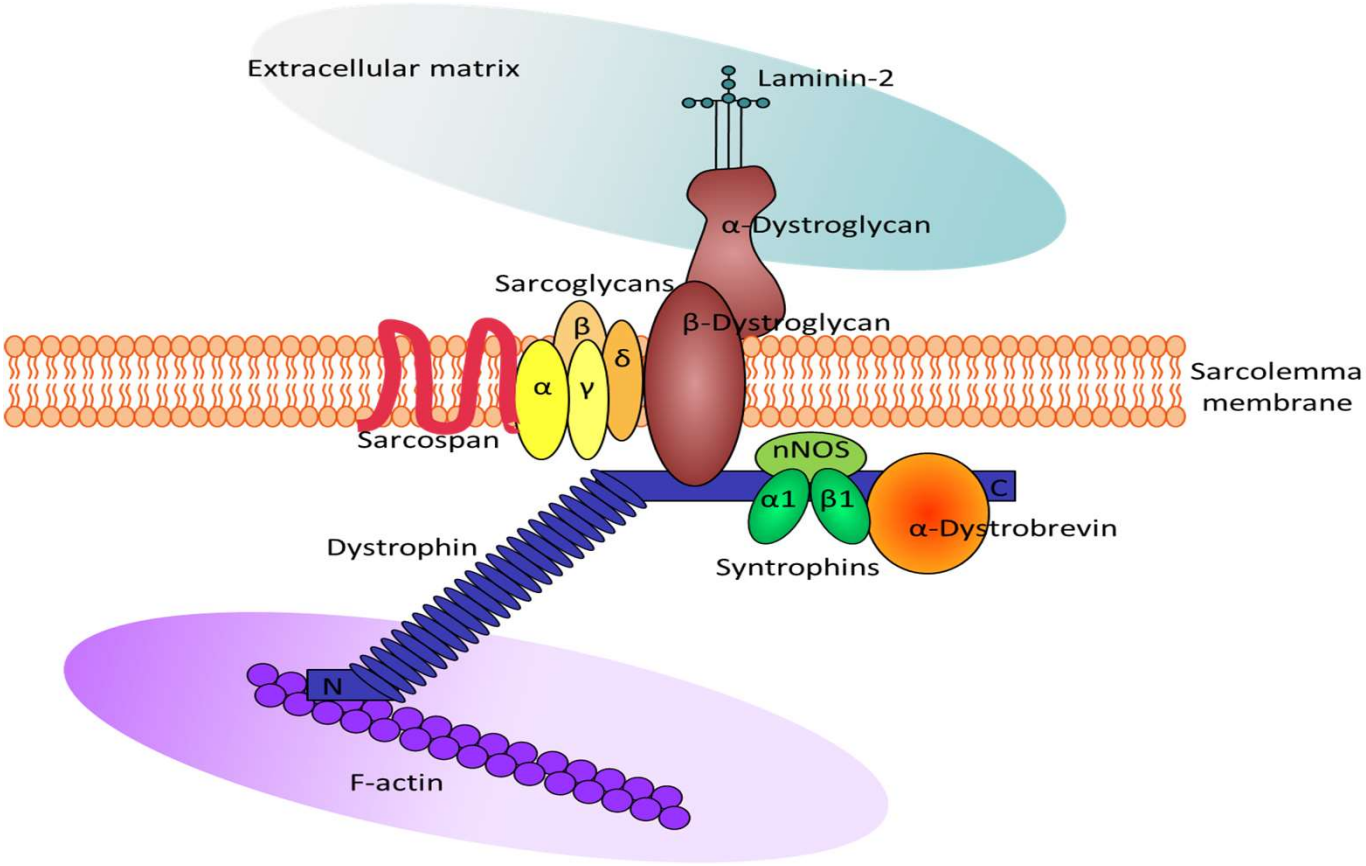
- Dystrophin *DMD* gene is the largest known human gene (2.4 Mb), containing 79 exons
- Nonsense or frame-shift mutations
- Hotspots: deletions between exons 45-55 and duplications between exons 2-10
- Thus these mutations lead to loss of dystrophin expression in the muscle fibres
- 1 in 3 cases is caused from a *de novo* mutation



Chamberlain, J. R. and J. S. Chamberlain (2017). Molecular Therapy 25(5): 1125-1131

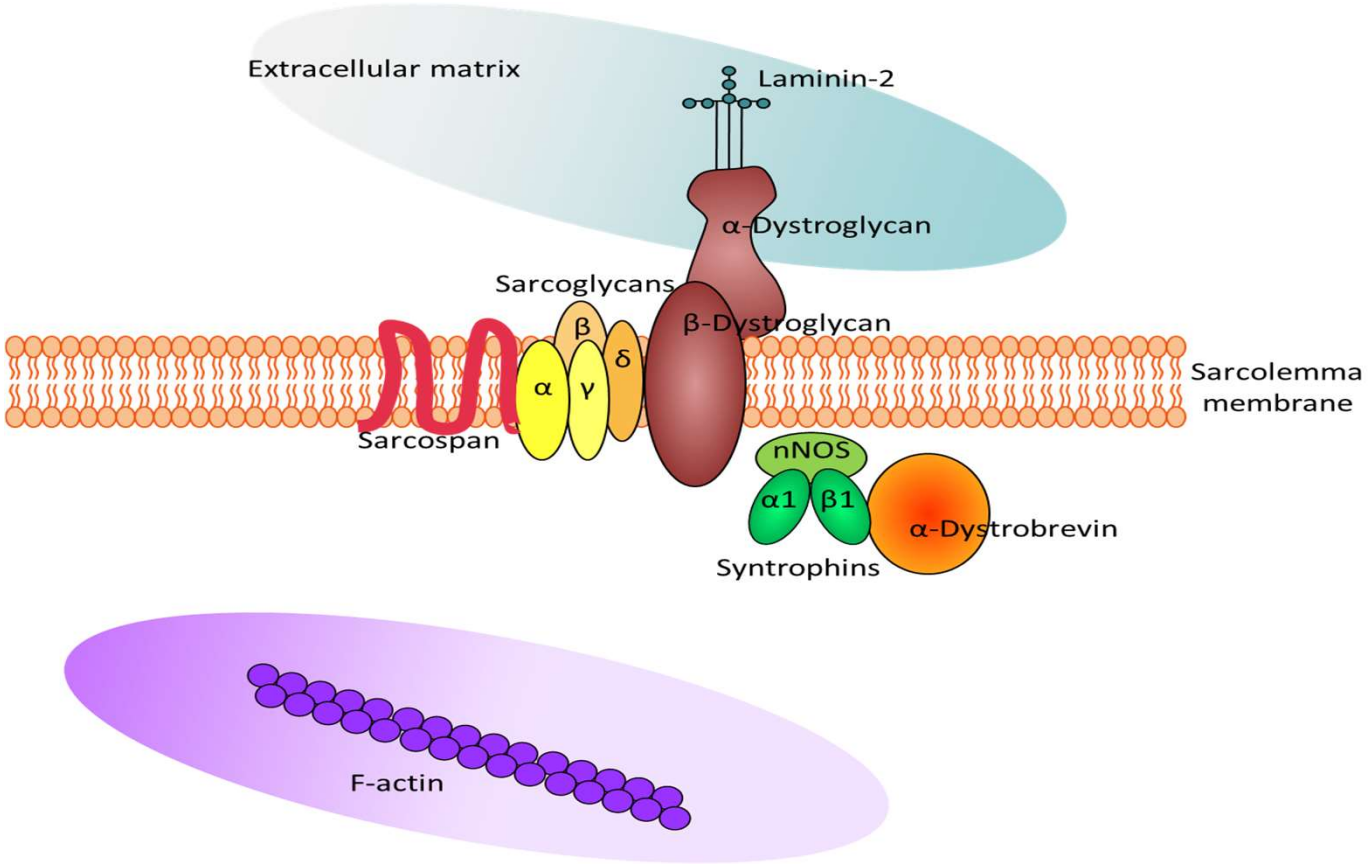
DMD protein

The dystrophin associated glycoprotein complex (DGC)



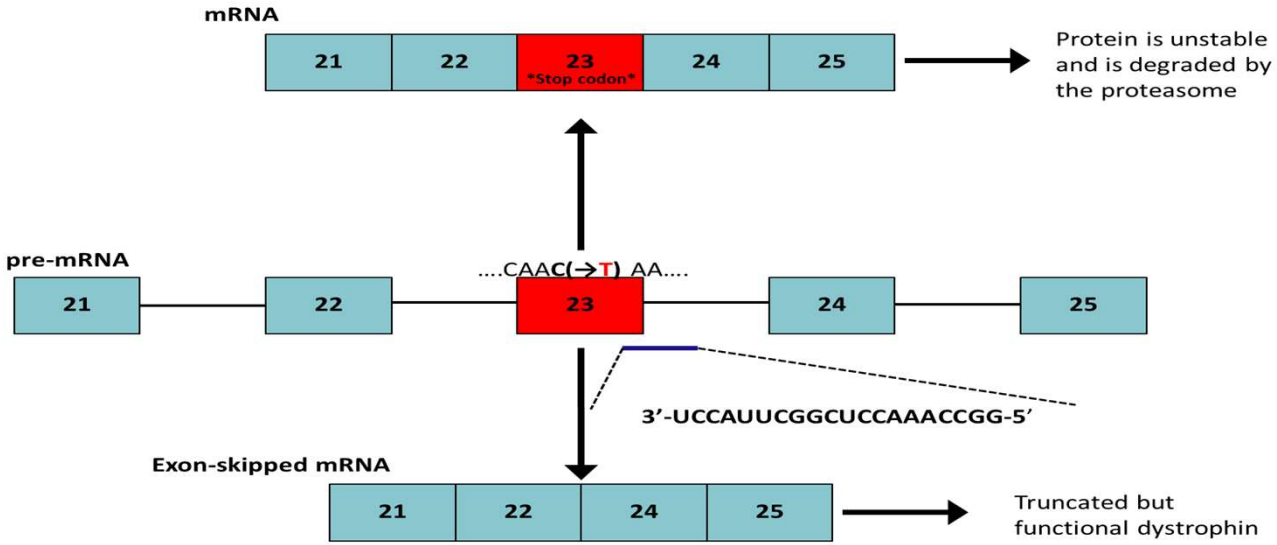
DMD protein

The dystrophin associated glycoprotein complex (DGC)



Potential Therapeutic Treatment

Exon skipping in the DMD (*mdx*) mouse model

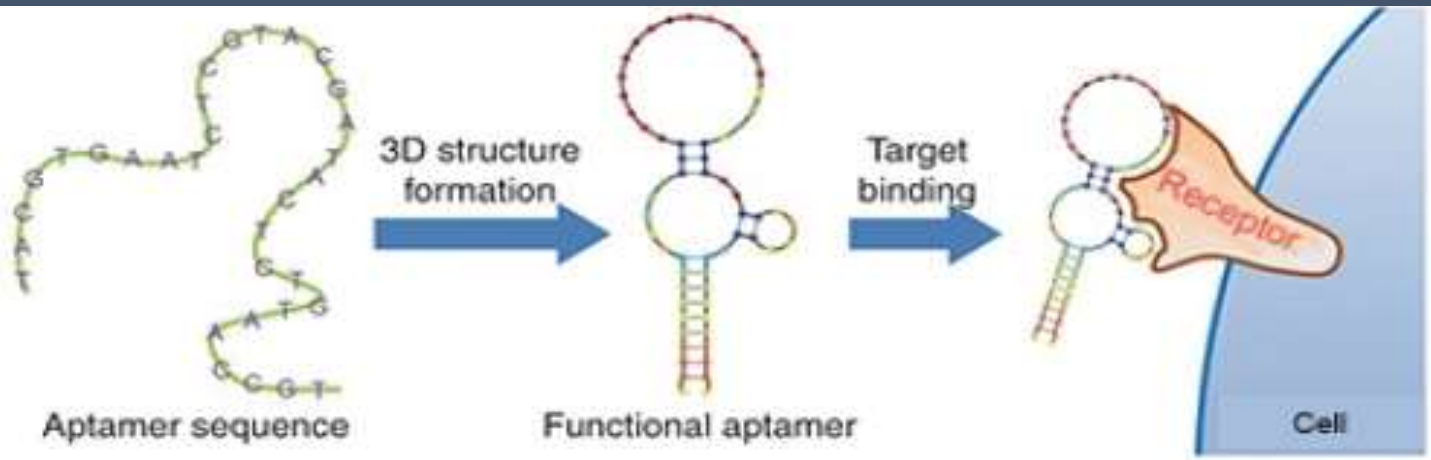


~ 83% of all DMD mutations could be treated by exon skipping

Delivery of AON in muscle

Aptamers

- Synthetic nucleic acid molecules designed to bind with high specificity and affinity to a selected target.
- Fold into unique three-dimensional structures.
- Systematic Evolution of Ligands by Exponential Enrichment (SELEX).
  - ✓ “Survival of the fittest”.
  - ✓ Has been modified in different ways for a number of applications.
  - ✓ Selective targeting of cells for the delivery of therapeutic molecules: siRNAs, miRNAs, chemotherapeutics and toxins.

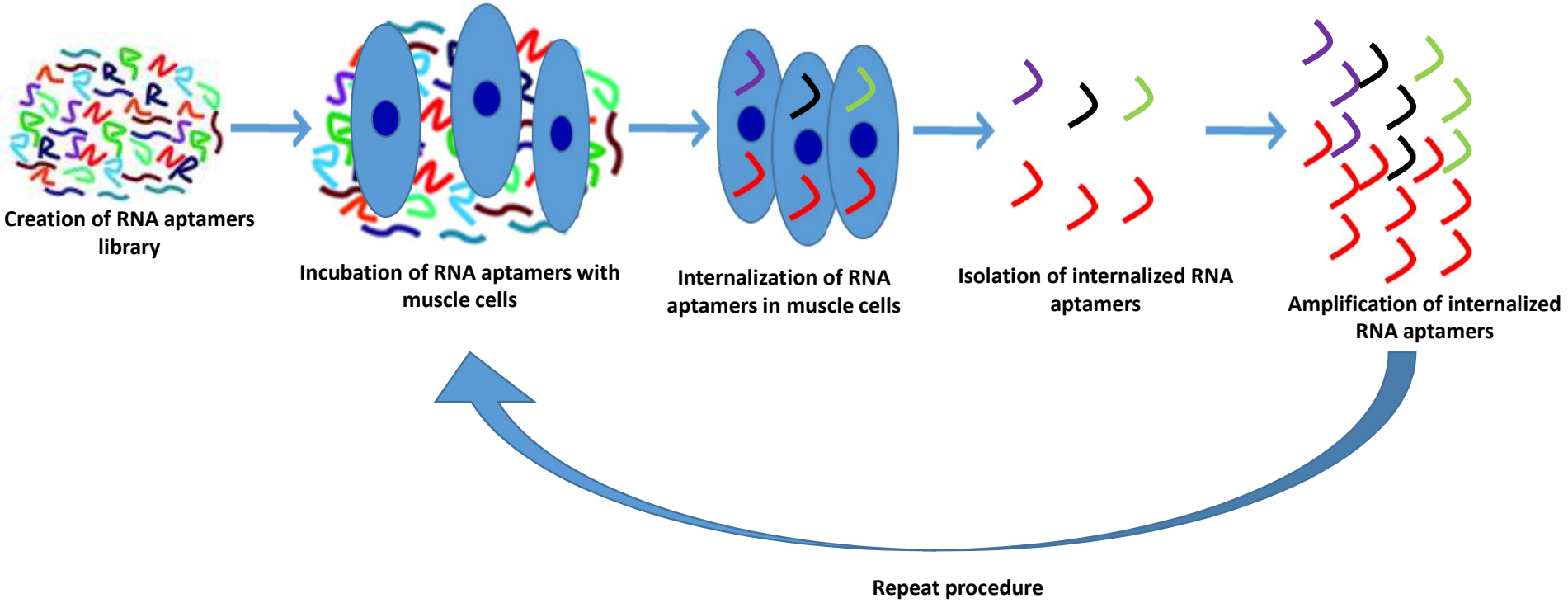


Romero-Lopez, C. and A. Berzal-Herranz (2017). "Aptamers: Biomedical Interest and Applications." Pharmaceuticals 10(1)

Aptamer Delivery of AON in muscle

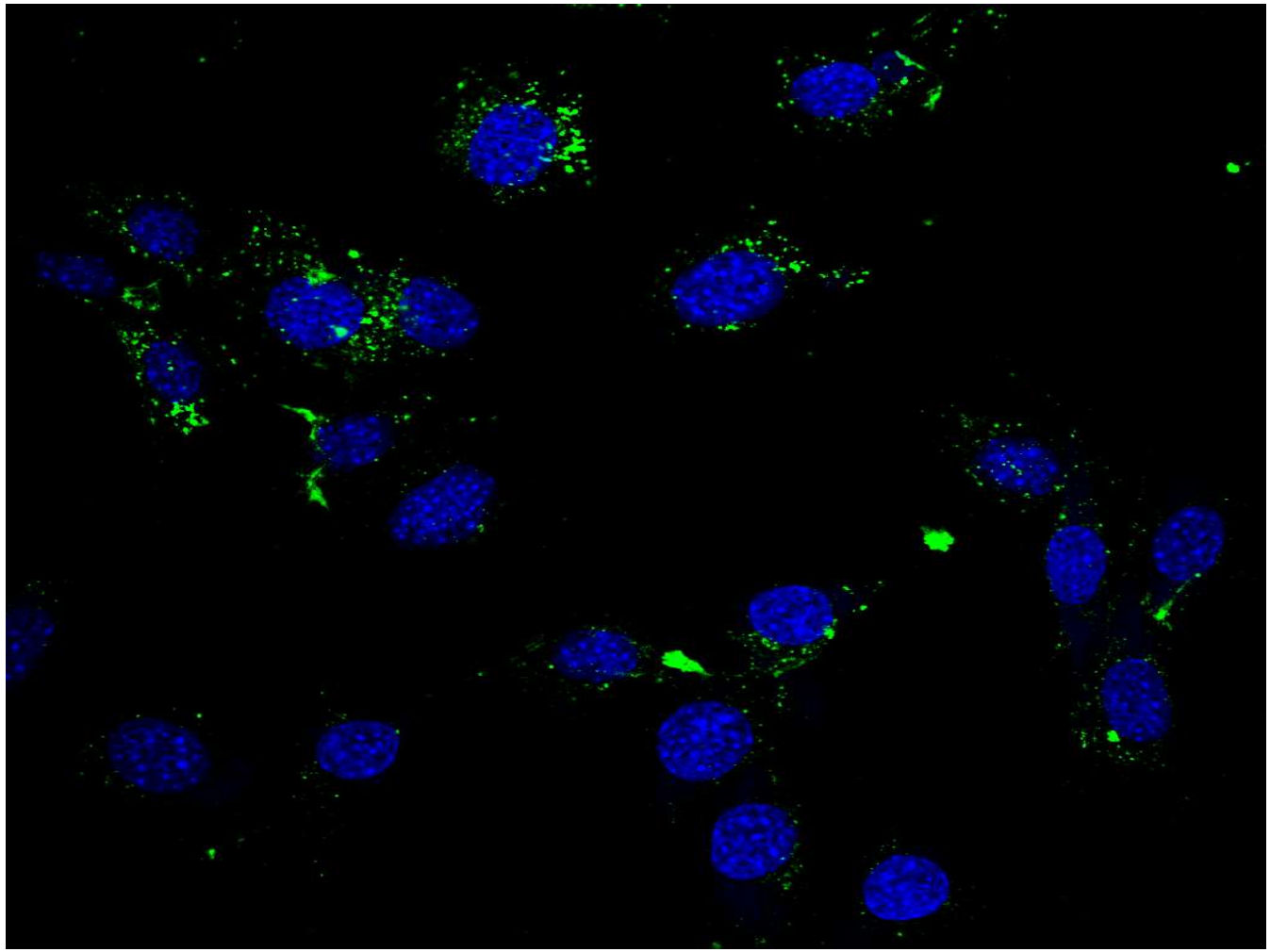
Cell-Internalizing SELEX

for skeletal muscle RNA aptamers



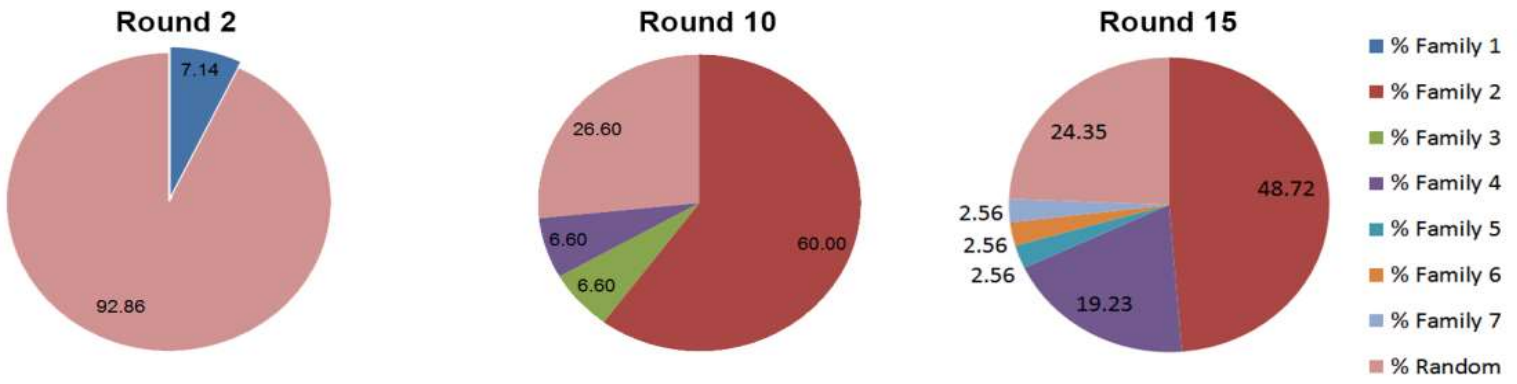
Philippou et. al. 2017 Molecular Therapy Nucleic Acids

Fluorescein labelled RNA aptamers pool (round 15) + DAPI



Round 15 pool / Nucleus

Aptamer convergence and alignment



Family 1 5'-GGGAAGAGAAGGACATATGAT **GGGGGGGAAGTGAGTACAGGAGGGCGCGAGTGTGCTGTAG** TTGACTAGTACATGACCACTTGA-3'

Family 2 5'-GGGAAGAGAAGGACATATGAT **CAGGAGCCGAGAACCGGTTGGTGGGTAATCCTGTTAGCGC** TTGACTAGTACATGACCACTTGA-3'

Family 3 5'-GGGAAGAGAAGGACATATGAT **CCGGTTTGGCTGAGCGTCGGCGCTCTGGAAGATGGCGTGG** TTGACTAGTACATGACCACTTGA-3'

Family 4 5'-GGGAAGAGAAGGACATATGAT **GTGGGTGGGAGTTGGTAGGGCGATGGGGAGAGGTGGCTGG** TTGACTAGTACATGACCACTTGA-3'

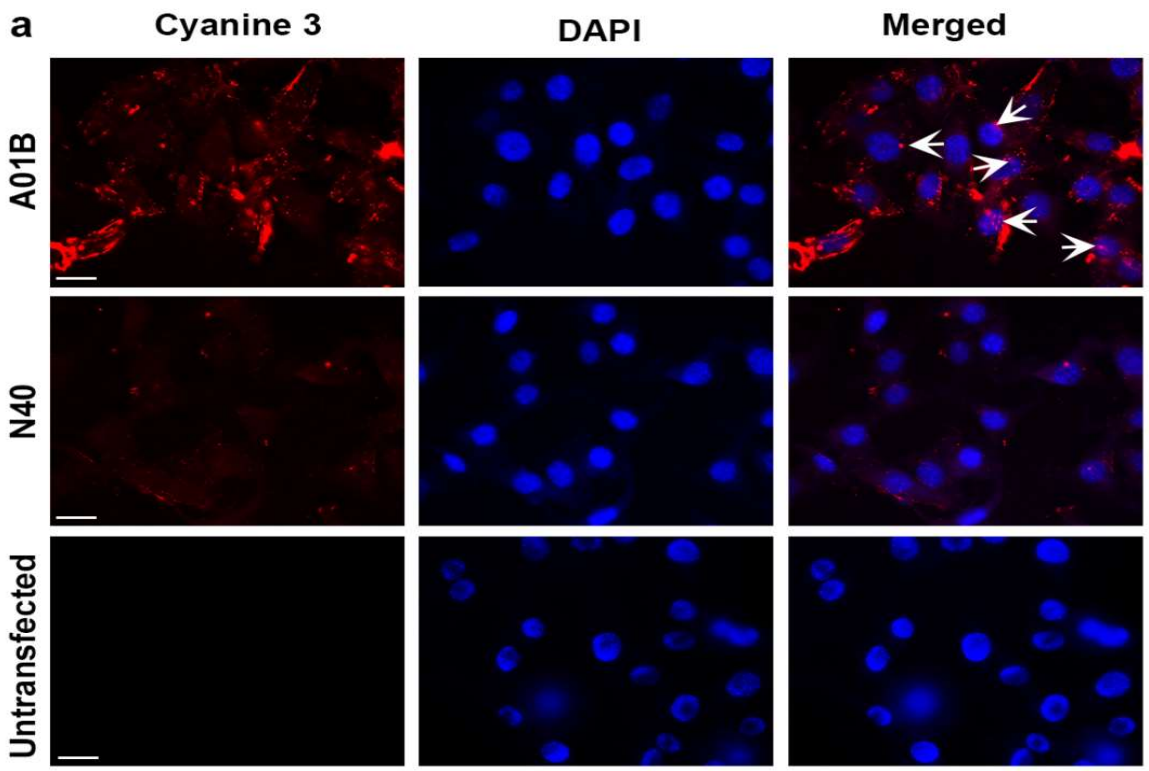
Family 5 5'-GGGAAGAGAAGGACATATGAT **CTATGGTGCGATAAAGGAGGCATCGCAATCGGTGGTGTGG** TTGACTAGTACATGACCACTTGA-3'

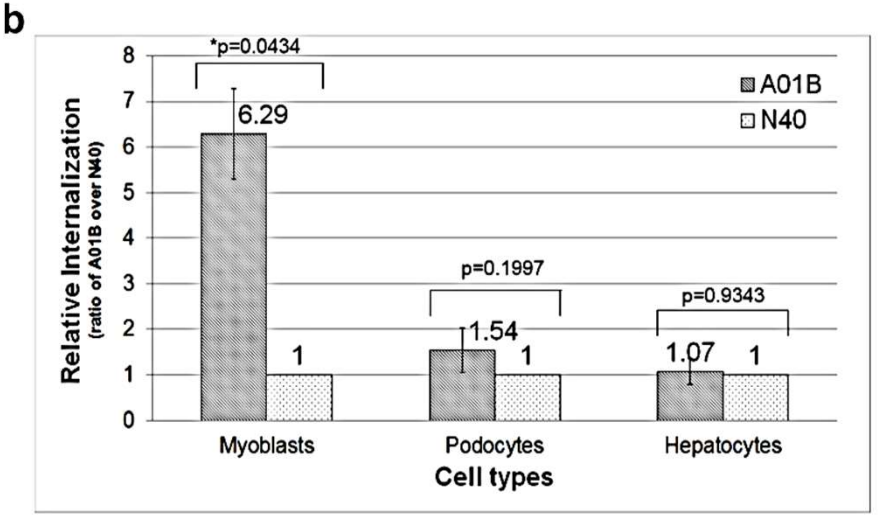
Family 6 5'-GGGAAGAGAAGGACATATGAT **GGGTAGGTTGGTAAGGTGGGGGGAGGAGGCAGTGTGGCAG** TTGACTAGTACATGACCACTTGA-3'

Family 7 5'-GGGAAGAGAAGGACATATGAT **TAGGGTGGCAGGTTAGGAGGGGAGGTAGGAGTGGGTGTGG** TTGACTAGTACATGACCACTTGA-3'

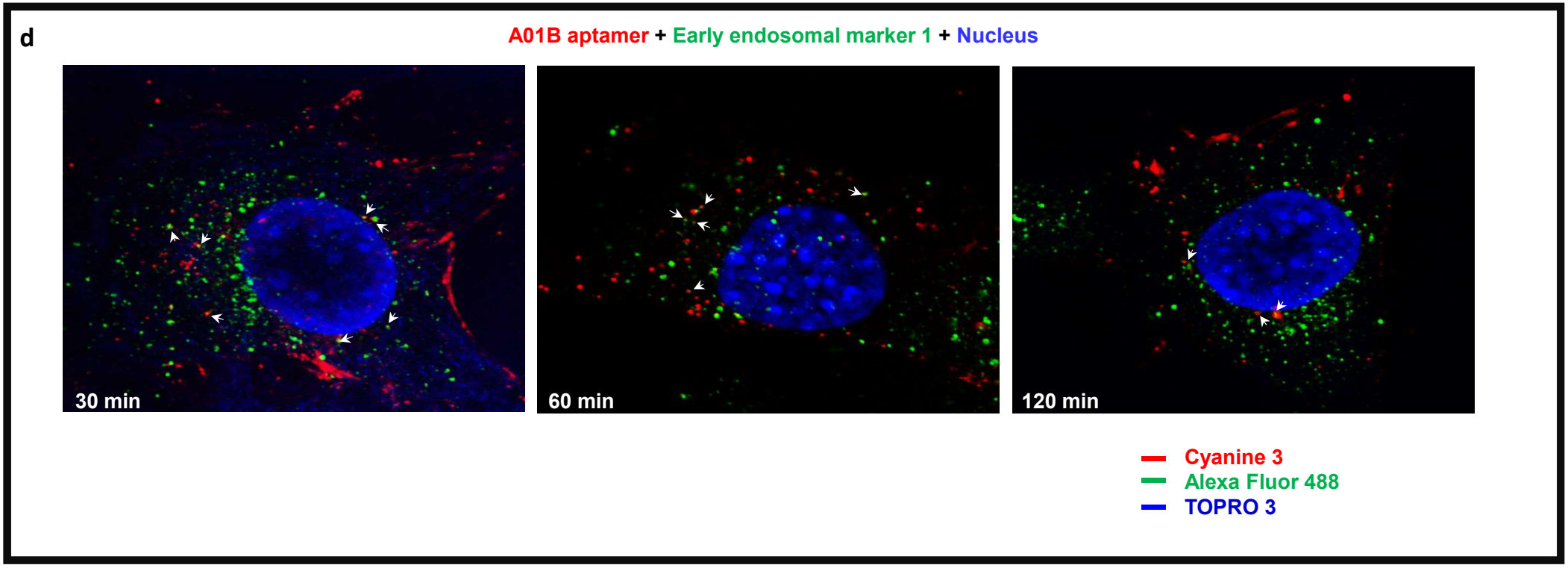
Random 5'-GGGAAGAGAAGGACATATGAT **NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN** TTGACTAGTACATGACCACTTGA-3'

Internalization and cellular localization of A01B RNA aptamer *in vitro*

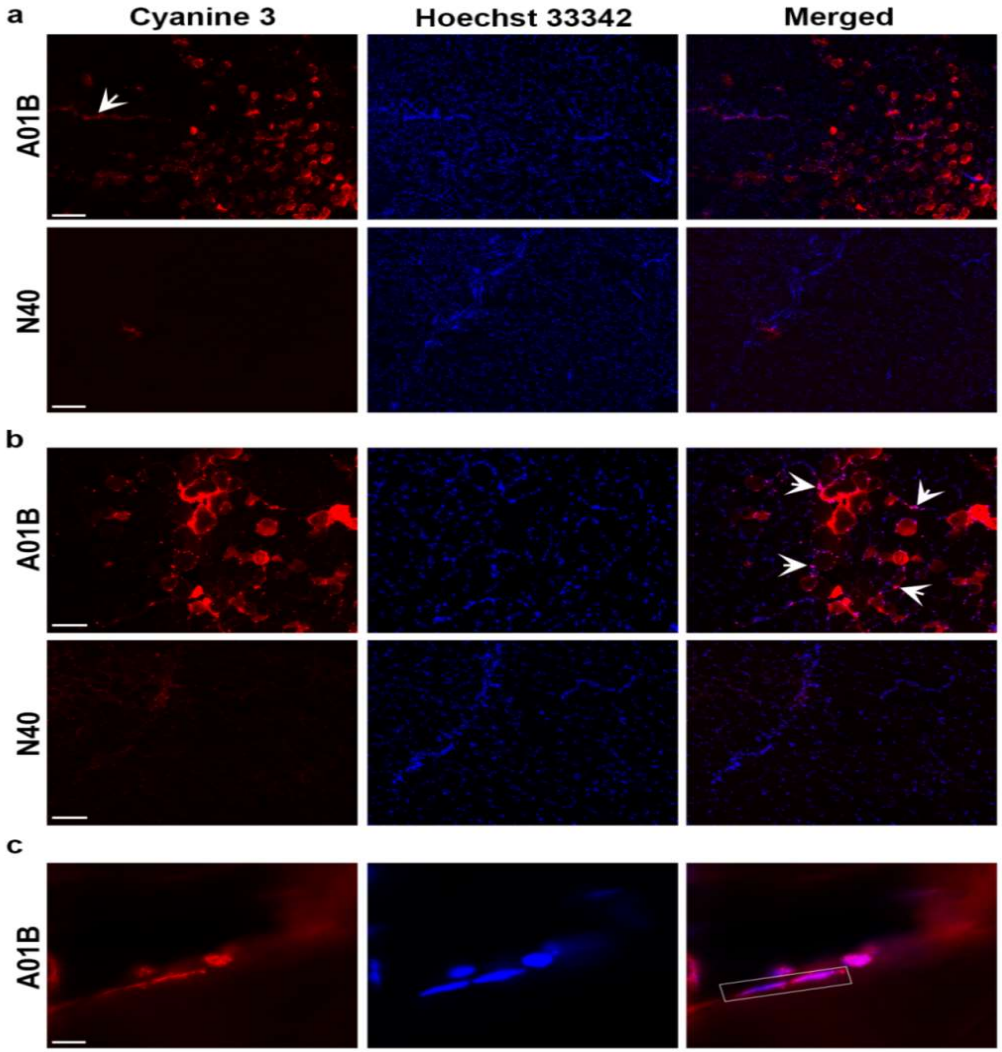




A01B RNA aptamer was found free from early endosomal compartments

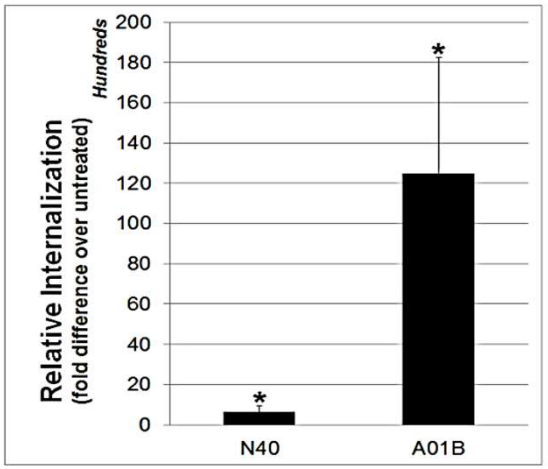


A01B RNA aptamer
internalizes efficiently into skeletal muscle



- Intramuscular injection of 100nM A01B RNA aptamer in TA muscle. Mice were sacrificed 1h post injection

d



Developing heart aptamers for AON delivery in DMD

Conclusions
& FUTURE DIRECTIONS

- Aptamers a novel approach to deliver specifically and efficiently to muscle
- First such aptamer discovered
- Incorporation of therapeutic oligonucleotides
- Specific targeting the heart muscle

Acknowledgements

THE CYPRUS INSTITUTE OF NEUROLOGY & GENETICS

❖ **Department of Molecular Genetics, Function & Therapy**

- Melina Christou
- Constantina Costi
- Pavlos Fanis
- Kristia Georgiou
- Demetris Koutalianos
- Andrie Koutsoulidou
- Nikolas Mastroyiannopoulos
- Stalo Mytidou
- Vassos Neocleous
- Styliana Philippou
- Leonidas Phylactou

FUNDING BODIES

THE A. G. LEVENTIS FOUNDATION · AFM Association Française contre les Myopathies · cost EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY · RESEARCH & INNOVATION FOUNDATION · TELETHON · CYPRUS SCHOOL of molecular medicine