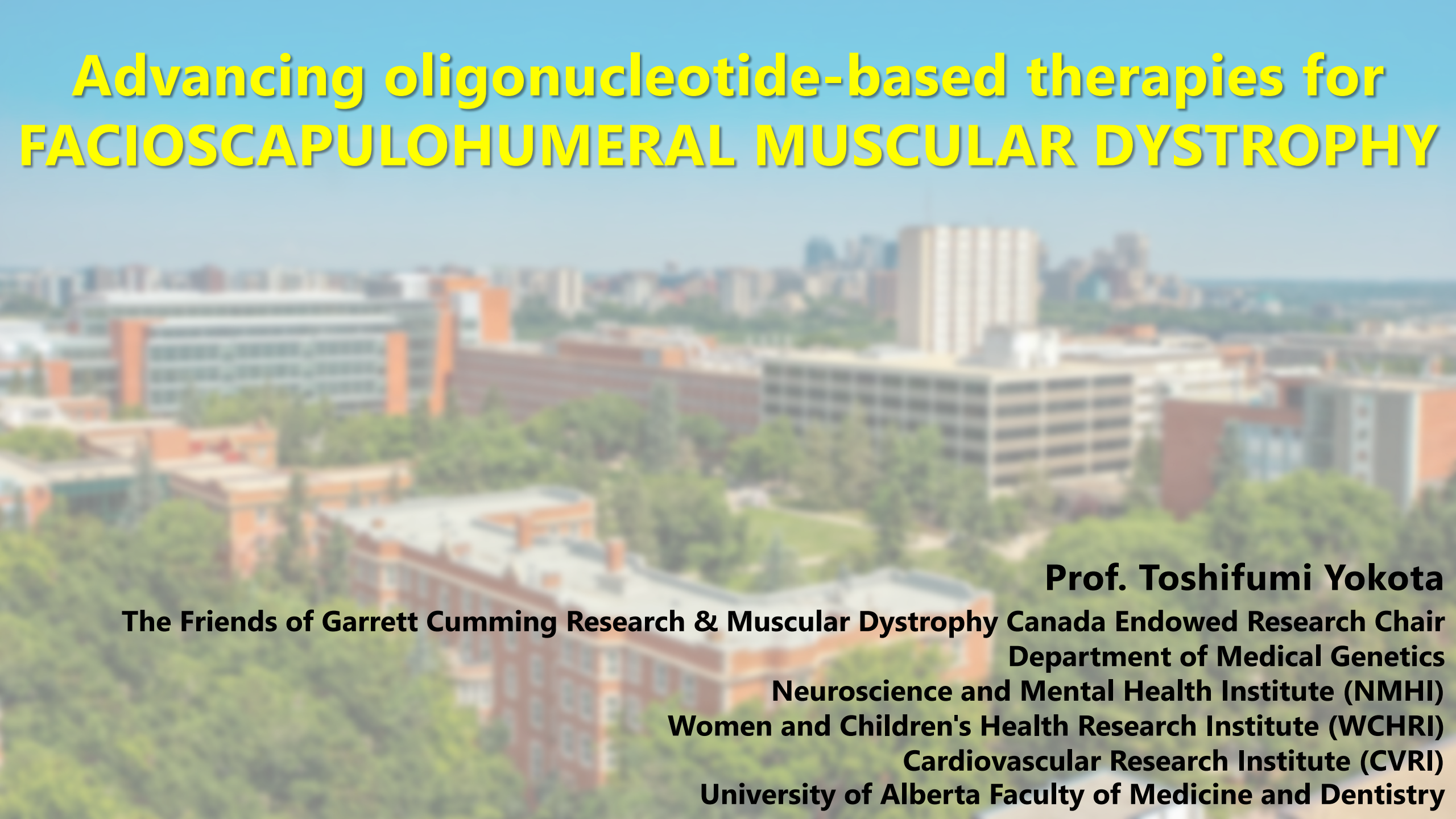
Advancing oligonucleotide-based therapies for FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY



Prof. Toshifumi Yokota

The Friends of Garrett Cumming Research & Muscular Dystrophy Canada Endowed Research Chair
Department of Medical Genetics
Neuroscience and Mental Health Institute (NMHI)
Women and Children's Health Research Institute (WCHRI)
Cardiovascular Research Institute (CVRI)
University of Alberta Faculty of Medicine and Dentistry

Relevant Financial Disclosure(s)

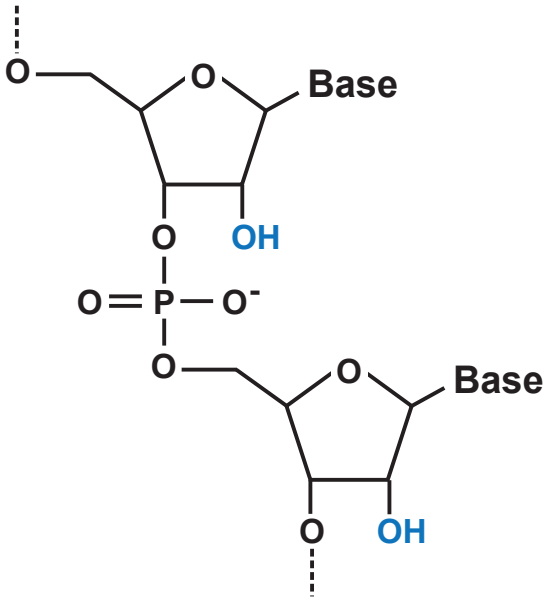
- Toshifumi Yokota, PhD

- OligomicsTx
  - Co-Founder, Shareholder

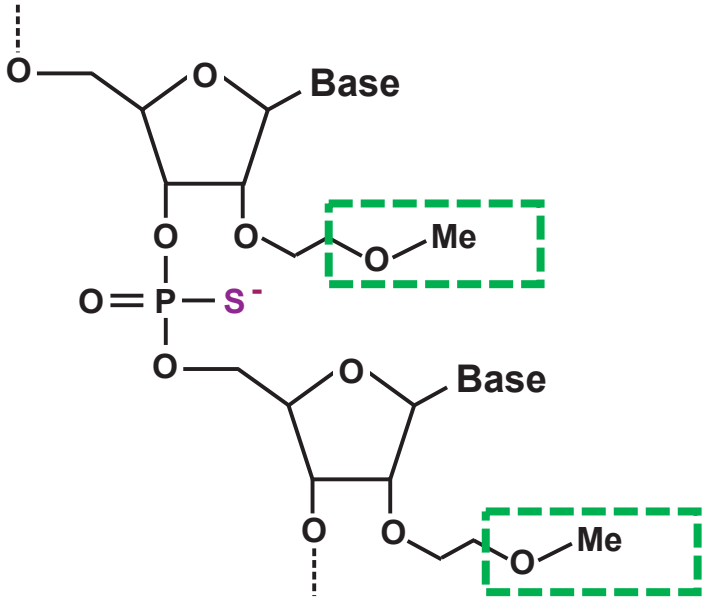
- Agada Bioscience
  - Consultant fee

Antisense oligonucleotide (ASO) therapy

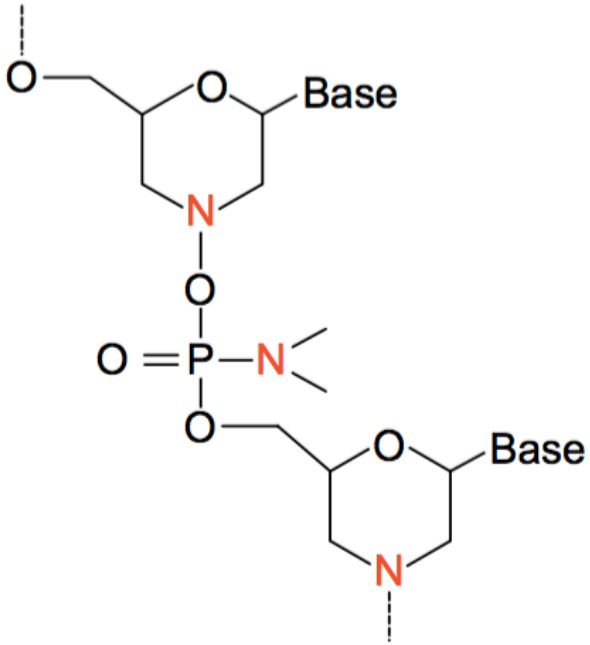
-An approach to fighting diseases using synthetic single-stranded DNA-like molecules targeting RNA
-ASOs usually consist of 15-30 nucleotides complimentary to target RNAs



RNA

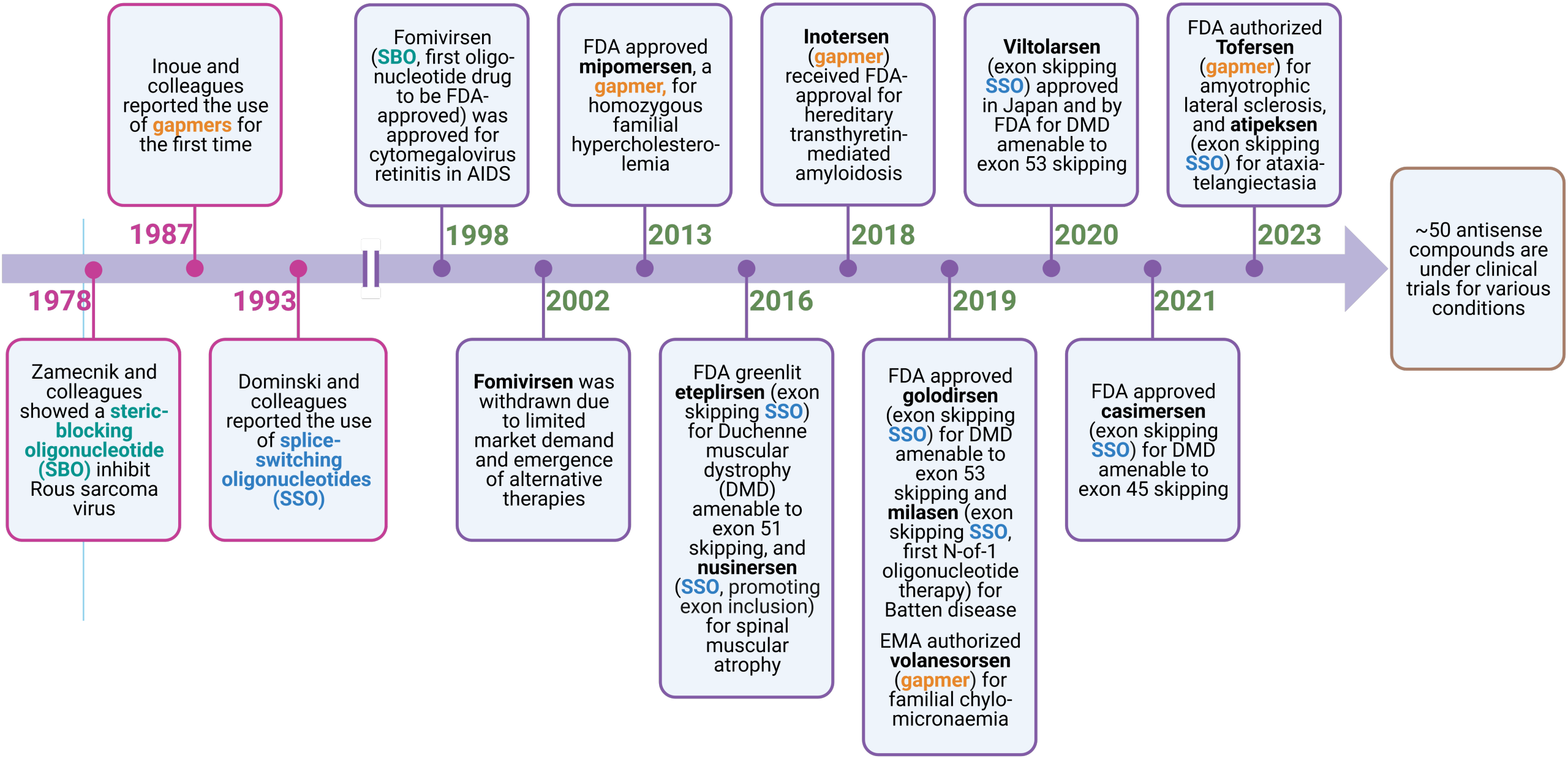


2’ MOE (Phosphorothioate)
2'-O-methoxyethyl-RNA

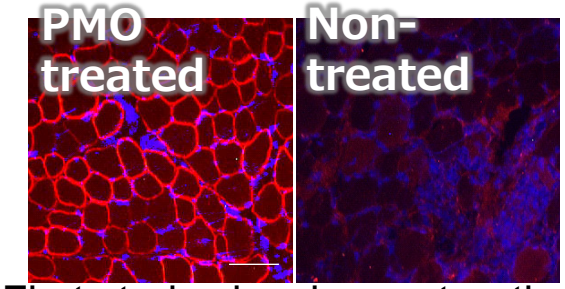


Morpholino / Phosphorodiamidate morpholino oligomer (PMO)

ANTISENSE OLIGONUCLEOTIDE (AO) THERAPY GETTING MOMENTUM SINCE 2013



Our study led to the development of an FDA-approved drug Viltolarsen :Translation from bench to bedside



First study showing restoration of dystrophin by exon skipping in a severe DMD animal model

Yokota et al. 2009

Clinical Trial Registration

US FDA Orphan Drug Designation

FDA Fast Track

FDA New Drug Application

Conditional Approval In Japan

FDA Approval

2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 | 2018 | 2019 | 2020

Collaboration with Nippon Shinyaku Started

PD/PK

ADME Toxicology

Quality Control

FIH Phase I in Japan (Clinician-led)

Phase I/II Trial (US/Canada/Japan)

Patient Registry (Remudy)

Patient recruitment

First DMD therapy that clearly restored dystrophin and improved muscle function in a clinical trial

Nippon Shinyaku

NCNP

Viltolarsen administered intravenously

Out-of-frame DMD mRNA

In-frame DMD mRNA

Clinical trials

Safety and pharmacokinetics

Molecular analysis

Functional assessment

FDA approval

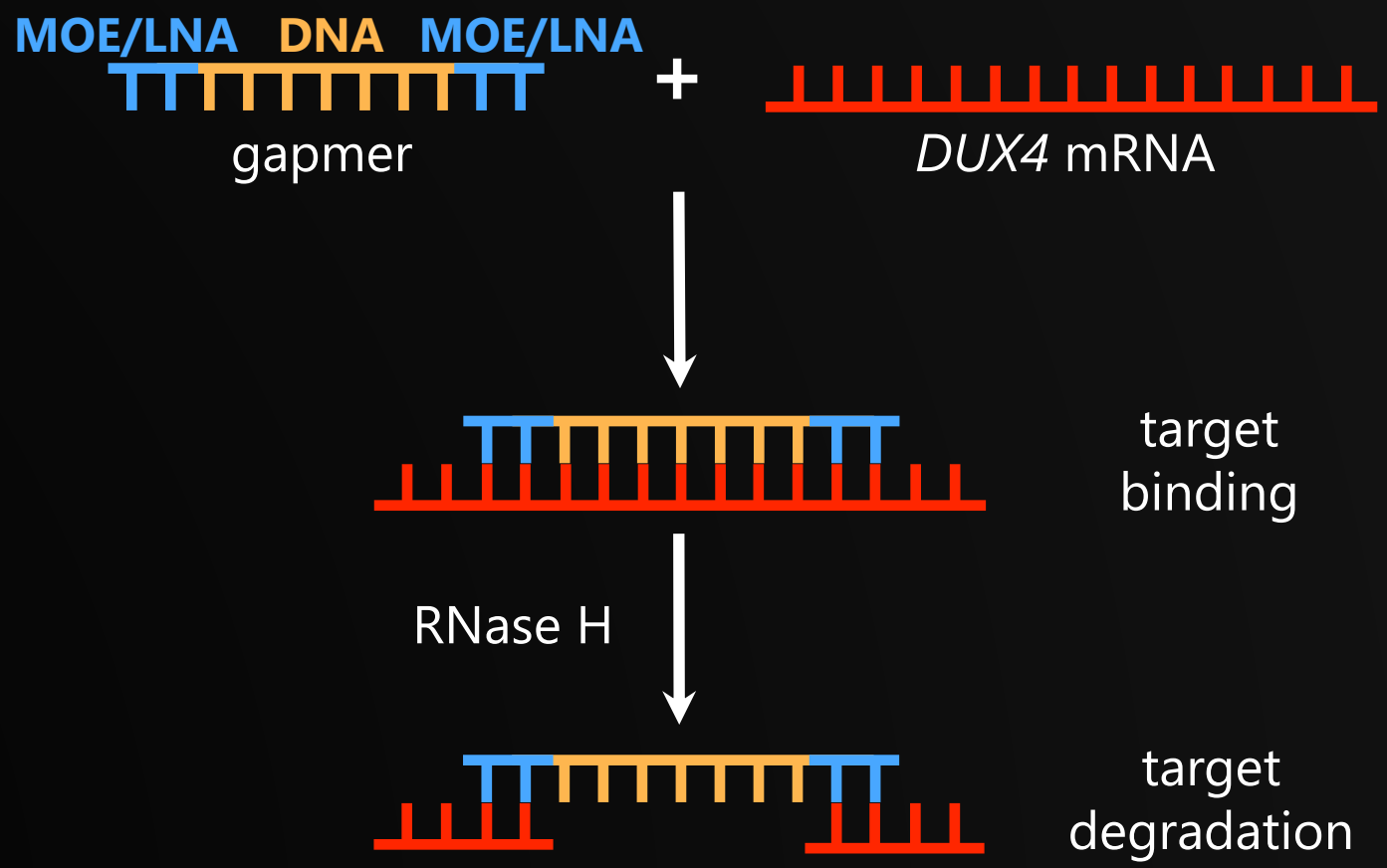
DUX4 is toxic for cells

- DUX4 in muscle cells cause
  - Muscle inflammation
  - Muscle wasting
  - Disrupted muscle development

- Only a trace amount of DUX4 is needed to cause FSHD
  - Expression of DUX4 in 1 out of 200–2000 cells can lead to FSHD

Using gapmers for gene knockdown

- Carefully designed gapmers targeting *DUX4*
- Gapmer hybridizes to the *DUX4* mRNA (target binding)
- *DUX4* mRNA:gapmer complex attracts RNase H
- RHase H degrades the mRNA:gapmer complex (target degradation)



Gapmer antisense technology: an unprecedented strategy to knock down DUX4 expression

PNAS Proceedings of the National Academy of Sciences of the United States of America

Inhibition of *DUX4* expression with antisense LNA gapmers as a therapy for facioscapulohumeral muscular dystrophy

Kenji Rowel Q. Lim[a], Rika Maruyama[a], Yusuke Echigoya[a,b], Quynh Nguyen[a], Aiping Zhang[c,d], Hunain Khawaja[c,d], Sreetama Sen Chandra[c,d], Takako Jones[e], Peter Jones[e], Yi-Wen Chen[c,f,1], and Toshifumi Yokota[a,g,1]

PNAS July 14, 2020 117 (28) 16509-16515; first published June 29, 2020 https://doi-org.login.ezproxy.library.ualberta.ca/10.1073/pnas.1909649117

Molecular Therapy

Original Article | Online Now

DUX4 transcript knockdown with antisense 2'-*O*-methoxyethyl gapmers for the treatment of facioscapulohumeral muscular dystrophy

Kenji Rowel Q. Lim • Adam Bittel • Rika Maruyama • Yusuke Echigoya • Quynh Nguyen • Yiqing Huang • Kasia Dzierlega Aiping Zhang • Yi-Wen Chen • Toshifumi Yokota • Show less

Published: October 14, 2020 • DOI: https://doi.org/10.1016/j.ymthe.2020.10.010

Our research hypothesis

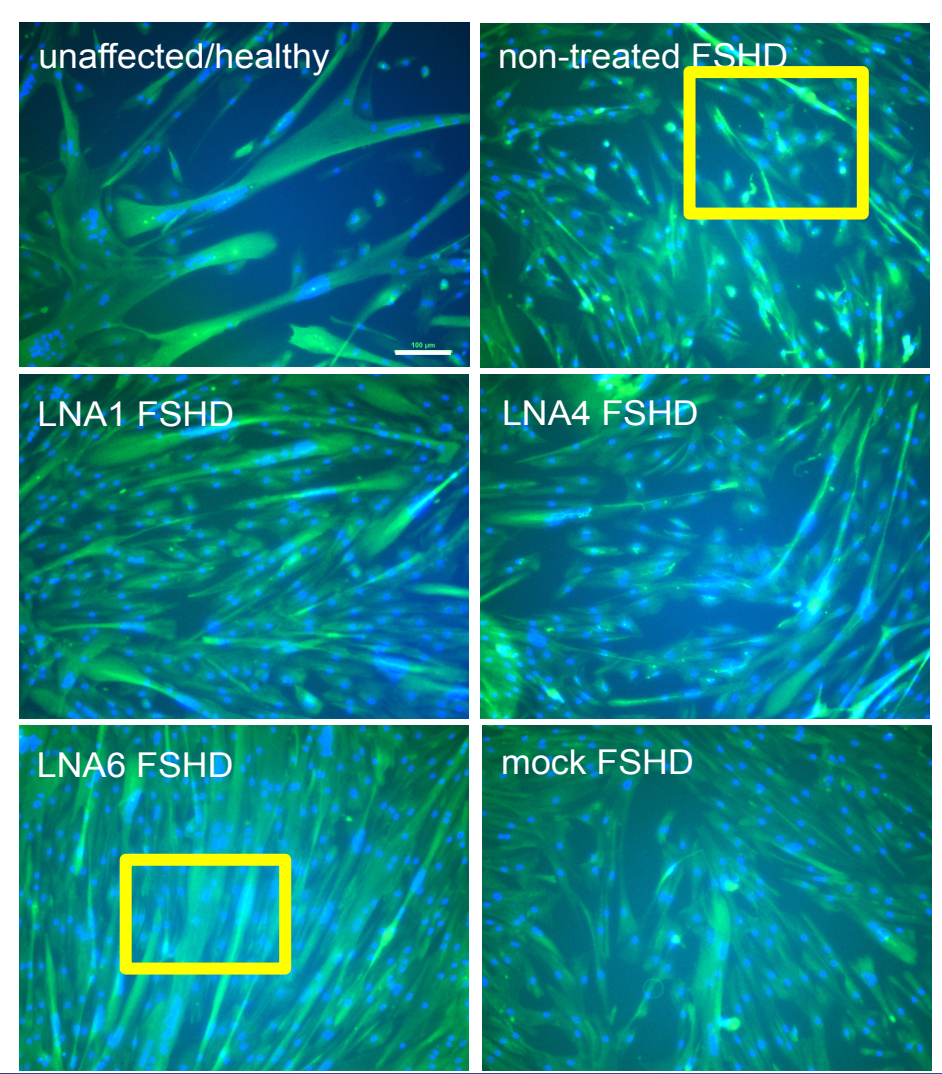
FSHD patient-derived muscle cells

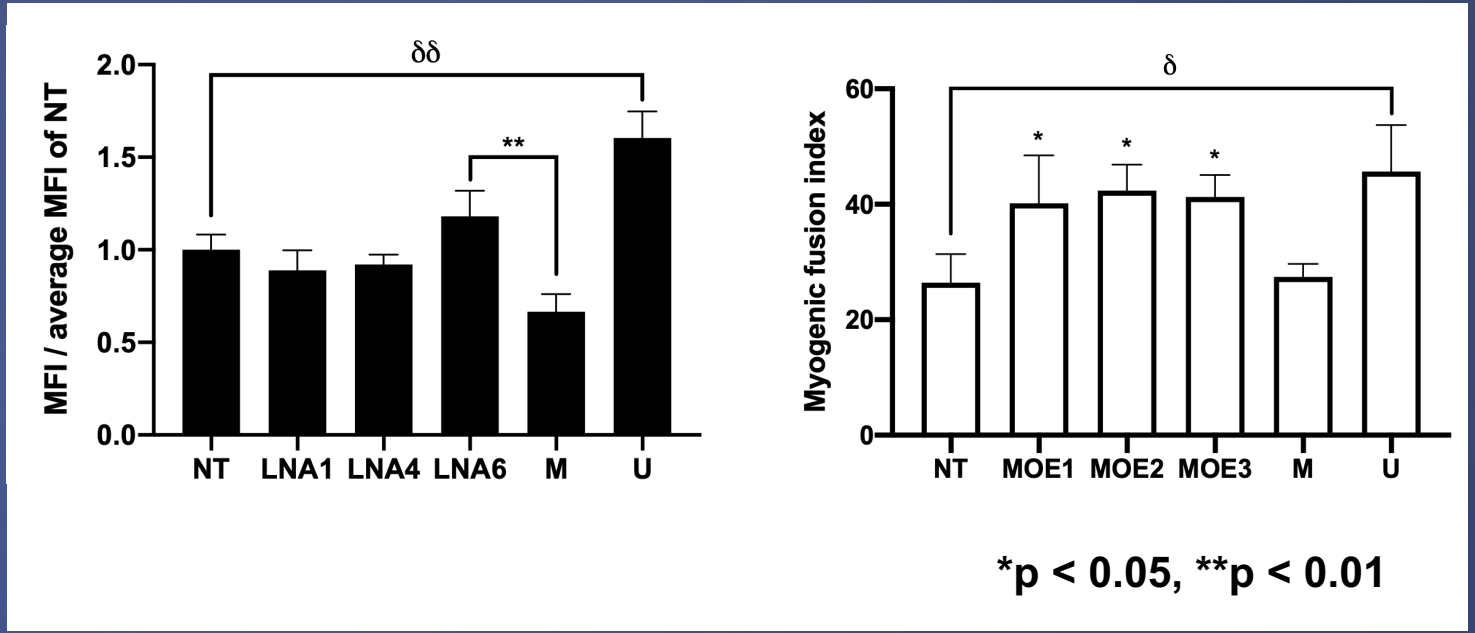
FSHD mouse model

→ *DUX4*?

We hypothesize that the use of our LNA and 2’-MOE gapmers will result in highly effective *DUX4* knockdown in FSHD-patient derived cell models and in a mouse of FSHD

LNA/2'-MOE gapmer treatment significantly improves muscle fusion in vitro

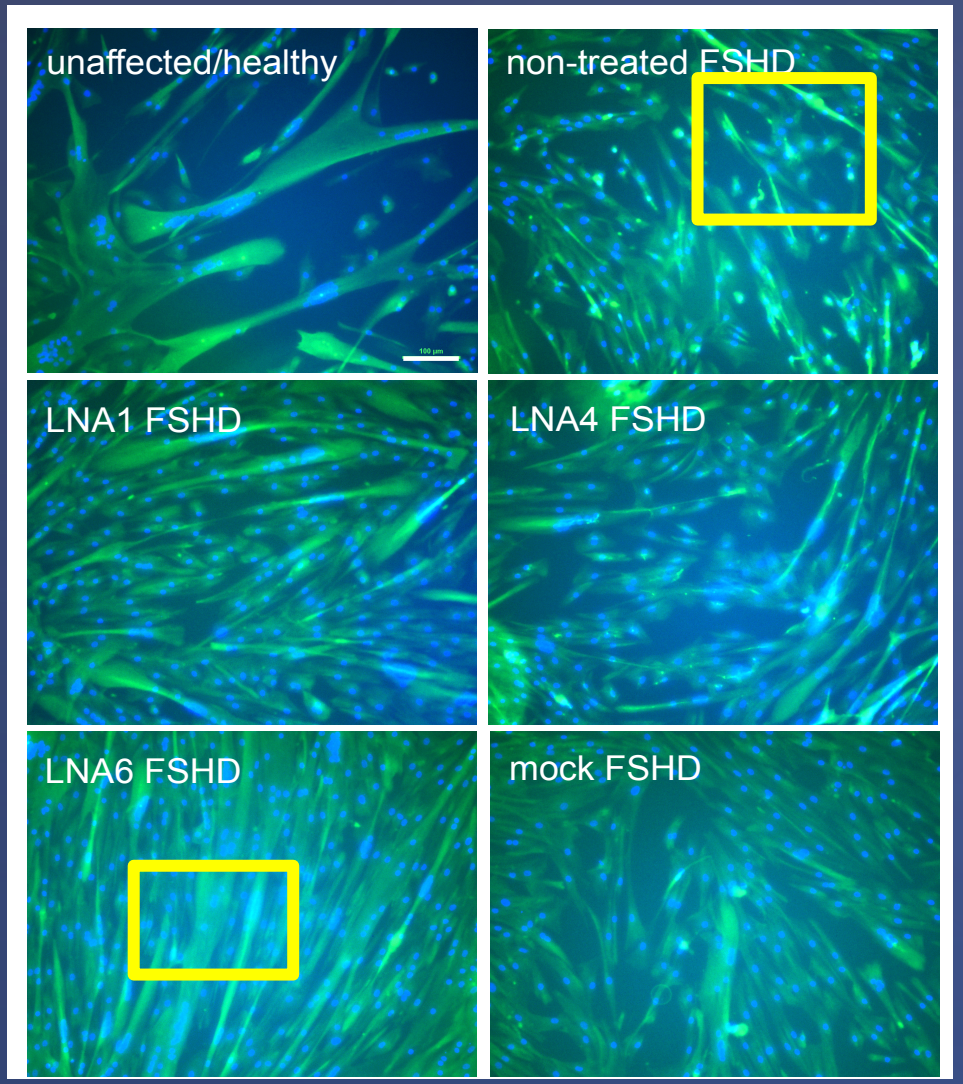


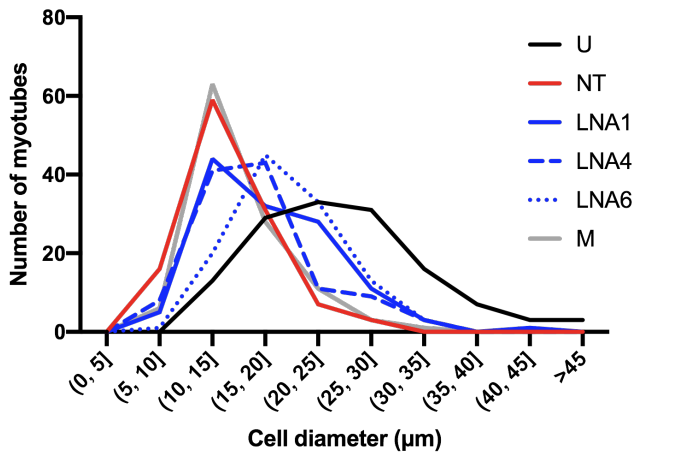


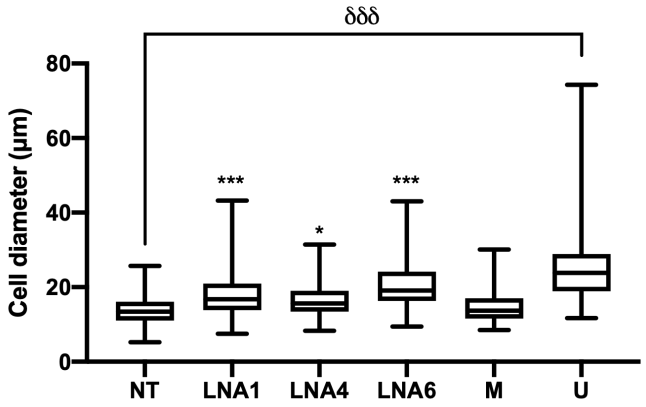
Myogenic fusion index (MFI) =
 # nuclei in myotubes / # total nuclei

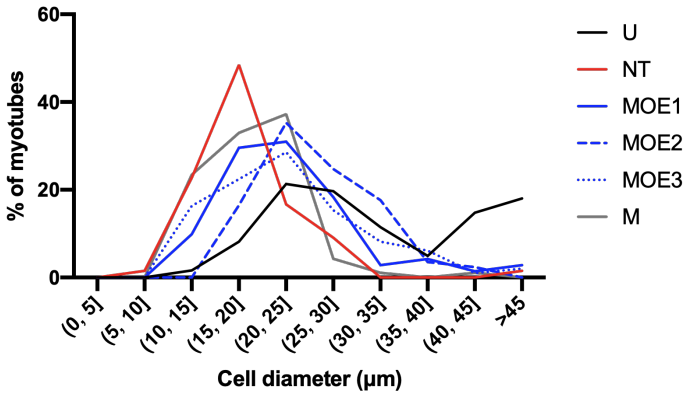
Lim et al. PNAS 2020

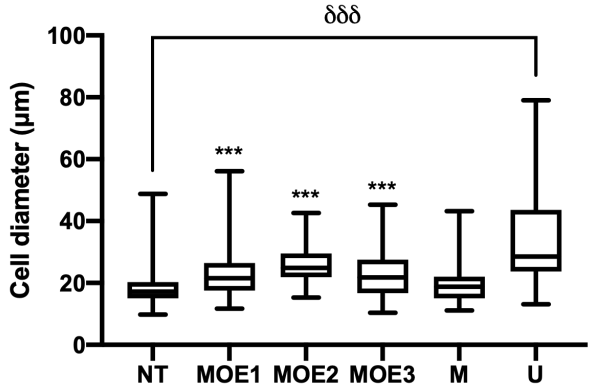
LNA/2’-MOE gapmer treatment significantly increases myotube diameters in vitro











*p < 0.05, **p < 0.01, ***p < 0.005

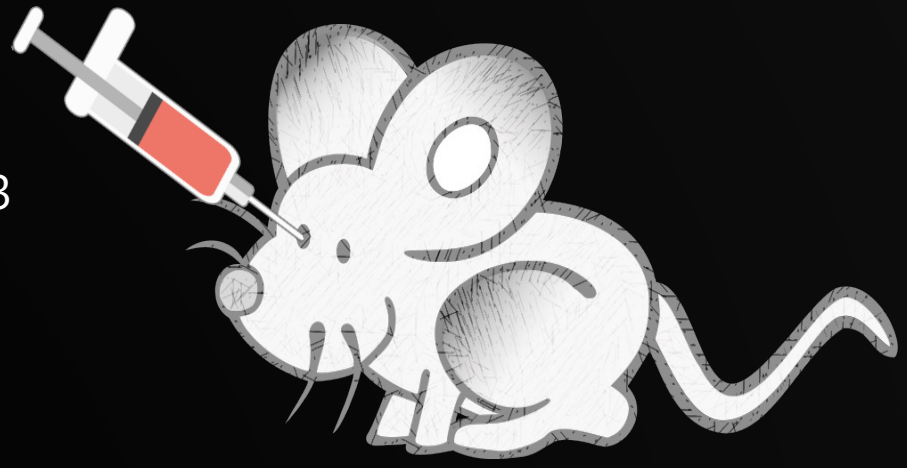
Lim et al. PNAS 2020

Lim et al. Mol Ther. 2021

Testing gapmers in an FSHD mouse model

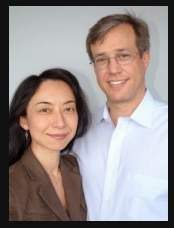
2-3 months old

LNA4/MOE3
20 µg



3x i.m.
every other day

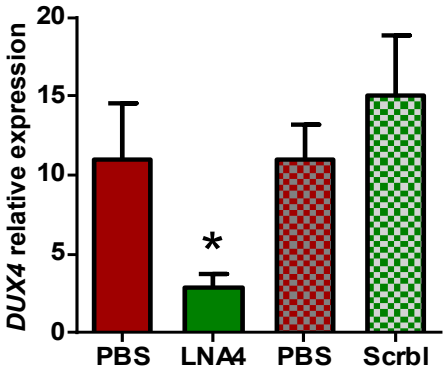
FLExDUX4 mice



Our LNA/2'-MOE gapmers reduce DUX 4 expression in vivo

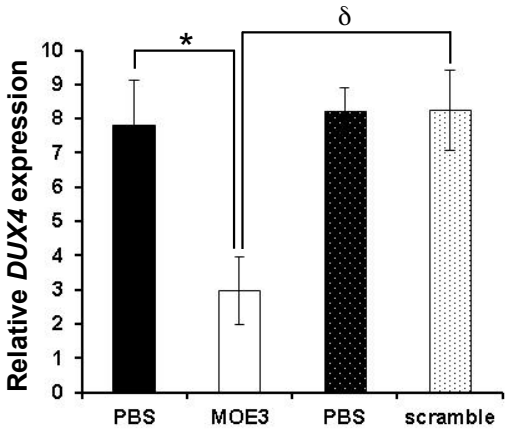
- Data show reduced DUX 4 expression in vivo and important muscle improvements

LNA gapmer



Lim et al. PNAS 2020

2'MOE gapmer

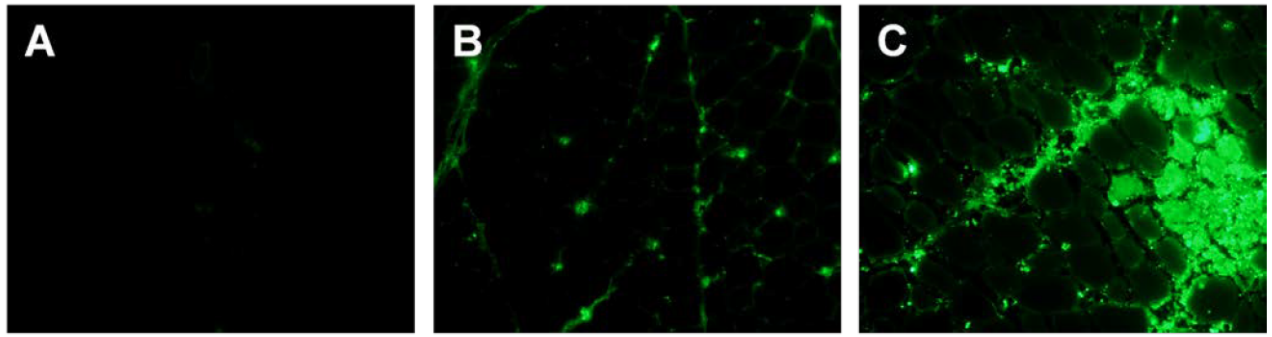


Lim et al. Mol Ther. 2021

FLExDUX4/+ mice
3 x i.m. of 30 μg LNA 4 / 20 μg MOE 3 into TA (every other day)
Collected 24 hours after the last injection

Scrbl: gapmer control with scrambled sequences
n=5 each

Away from injection site **injection site**



PBS **LNA** **LNA**

interstitial space (IS) **IS + myofibers**

7-week old FLExDUX4/+ mice,
1x i.m. of 30 mg Fluorescein-LNA 4 into TA
Collected 24 hours after the injection

Lim et al. PNAS 2020

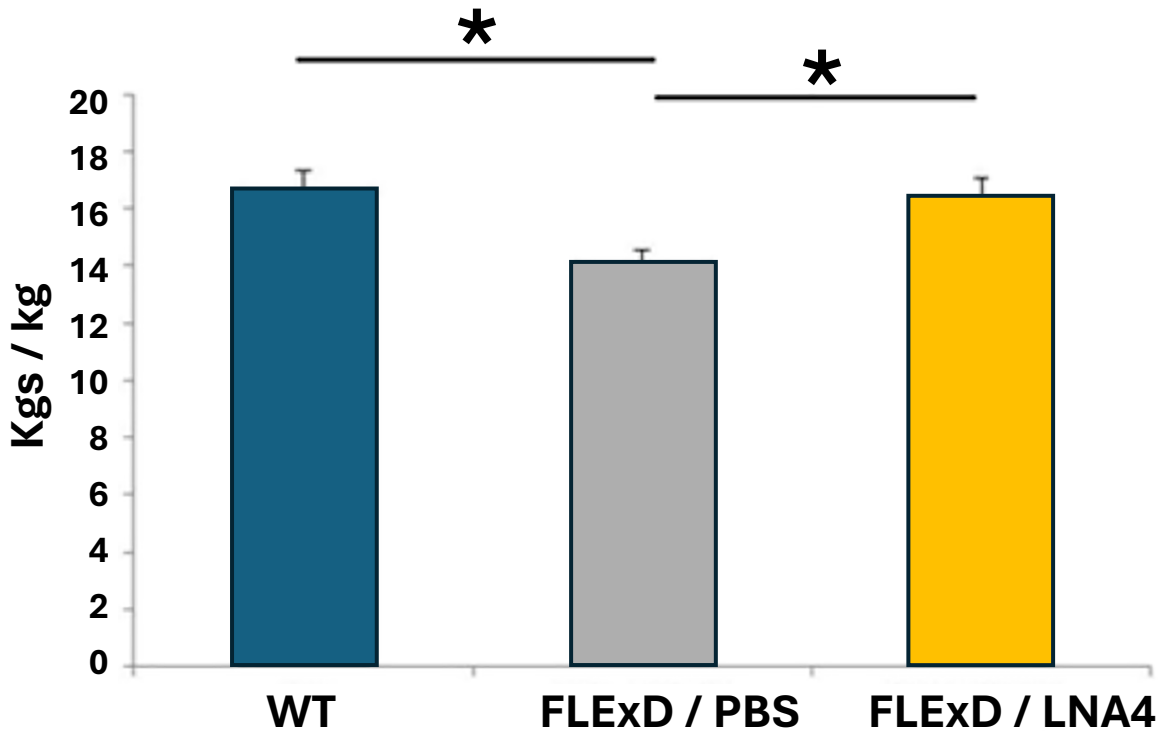
LNA gapmers improve muscle function of FSHD model mice

FLEx-DUX4/+ mice

Muscle Collection

Grip Strength Testing

Week 10



FLExDUX4/+ mice

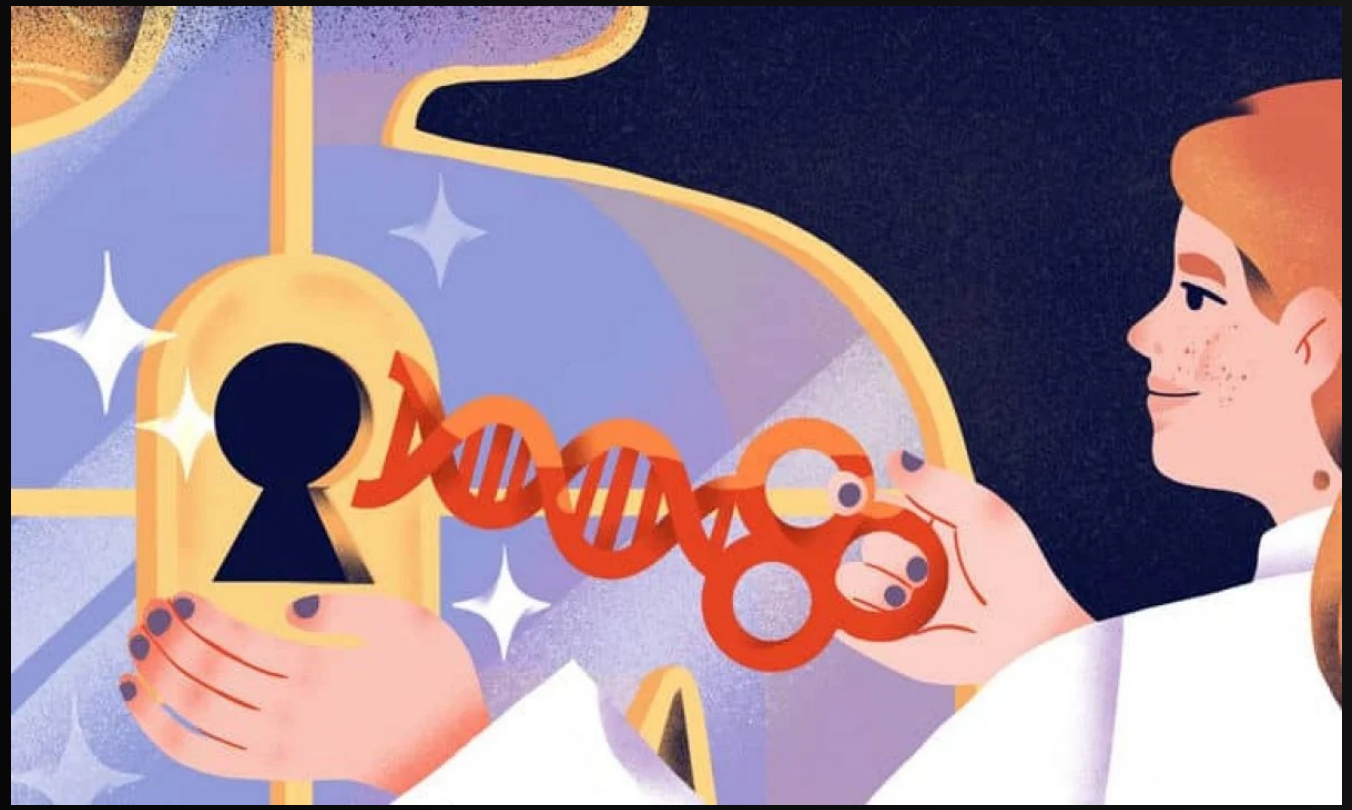
LNA gapmer 4
Subcutaneous injection (20mg/kg)
Twice a week for 10 weeks

N=5, *p< 0.05

US Patent Number 11,518,955
Issued December 6, 2022
Yokota T et al.

Conclusions and Future Work

- LNA and 2’-MOE gapmers effectively knock down ***DUX4***
- Carrier-based delivery of the gapmers are being tested



Acknowledgements

Kenji Rowel Q. Lim, Tejal Aslesh, Rika Maruyama, PhD
Yusuke Echigoya, PhD, Stanley Woo, Saeed Anwar
Quynh Nguyen
Department of Medical Genetics, University of Alberta

Hunain Khawaja, Adam Bittel, Sreetama Sen Chandra, Yi-Wen Chen
Center for Genetic Medicine Research, Children's National Health System

Takako Jones, Peter Jones
Reno School of Medicine, University of Nevada

Charles Emerson Jr., Jennifer Chen
Wellstone Program of the University of Massachusetts Medical School

Acknowledgements

THANK YOU