Antisense oligonucleotide therapeutics for neurological and neuromuscular disorders

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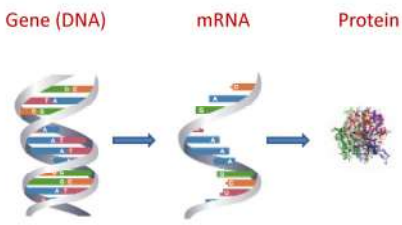
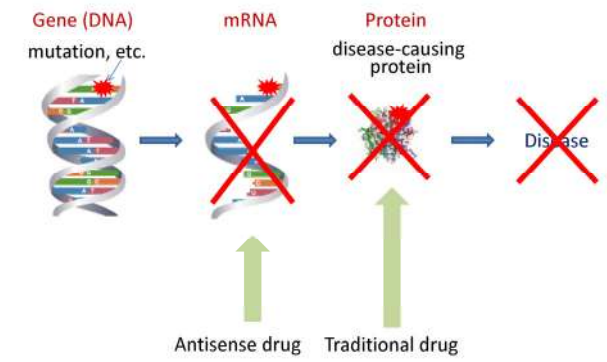
Agenda

- Overview of antisense oligonucleotides (ASOs) technology
- Treatment strategy for neurological/neuromuscular diseases
  - — Duchenne Muscular Dystrophy, Spinal Muscular Atrophy
  - — Myotonic Dystrophy
    Huntington Disease, ALS, Familial Amyloid Polyneuropathy
  - — Parkinson Disease
- Additional advantage of ASOs therapy
  - — Trinucleotide Repeat Expansion Disorders

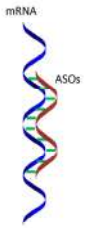
Central Dogma



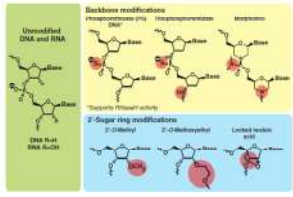
Antisense drug technologies



Antisense oligonucleotides (ASOs)

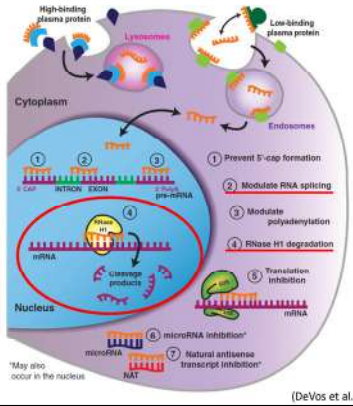


- Short oligonucleotides (12-25 nt) specifically designed to bind a complementary area of target RNA
- Modulate target RNA expression
- Chemically modified to increase binding affinity, stability, and tissue uptake



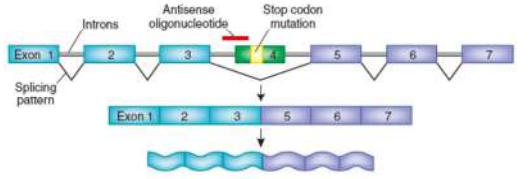
(DeVos et al. *Neurotherapeutics*, 2013)

ASOs mechanisms of action



(DeVos et al. *Neurotherapeutics*, 2013)

Splicing modulation by ASOs

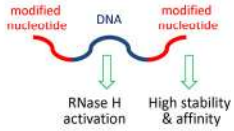


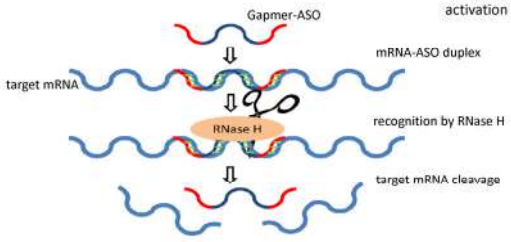
(Chamberlain et al. *Nature Med* 2010)

Target mRNA degradation by ASOs

Gapmer-ASO

- Chemically modified nucleotide (MOE, LNA, cEt, etc.) sequences at ends
- DNA sequence in middle
- Phosphorothioate throughout





- Overview of Antisense Oligonucleotides (ASOs) Technology
- Treatment strategy for neurological/neuromuscular diseases
  - — Duchenne Muscular Dystrophy, Spinal Muscular Atrophy
  - — Myotonic Dystrophy
    Huntington Disease, ALS, FAP
  - — Parkinson Disease
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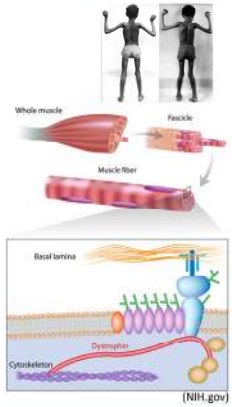
  - — Trinucleotide Repeat Expansion Disorders

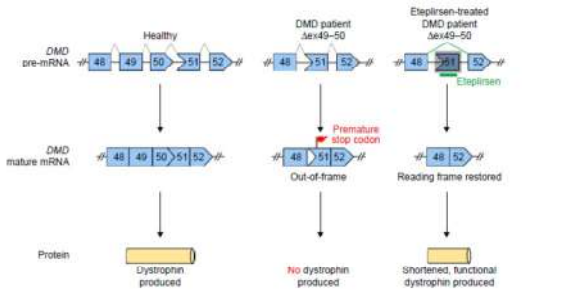
Duchenne Muscular Dystrophy (DMD)

- Prevalence of 1:3500 boys
- Presentation
  - — onset: 3-5 years old
  - — progressive weakness
  - — cardiomyopathy
  - — respiratory failure
- Caused by lack of Dystrophin protein
  (Dystrophin gene deletion, mutation, etc.)



(NIH.gov)

Exon skipping therapeutics for DMD

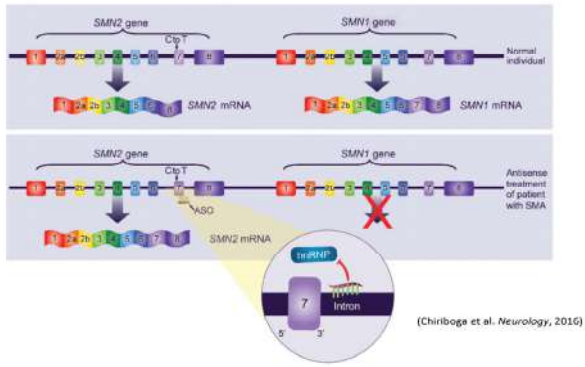


(Lim et al. *Drug design, development and therapy*, 2013)

Spinal Muscular Atrophy (SMA)

- Autosomal recessive disorder (prevalence of 1:100,000)
- Age of onset
  - SMA type 1 (acute infantile)
  - SMA type 2 (chronic infantile)
  - SMA type 3 (chronic juvenile)
  - SMA type 4 (adult onset)
- Progressive weakness due to damage of lower motor neuron
- Caused by mutations of *Survival motor neuron* (*SMN1*) gene

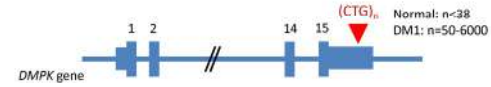
Splicing modulation for SMA



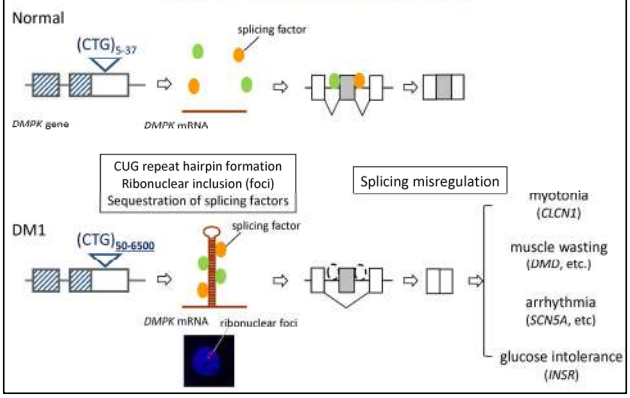
(Chiriboga et al. *Neurology*, 2016)

Myotonic Dystrophy type 1 (DM1)

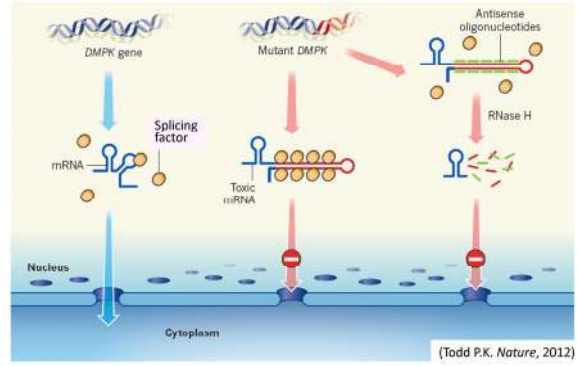
- Most common type of muscular dystrophy in adults
- Multi-systemic disease
  - myotonia, progressive muscle wasting
  - cardiac conduction defects, arrhythmia
  - cognitive impairment
  - glucose intolerance, etc.
- Caused by CTG repeat expansion in 3′ UTR of *DMPK* gene



Pathomechanism in DM1
(toxic RNA and splicing abnormality)



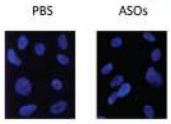
Toxic RNA degradation by ASOs



(Todd P.K. *Nature*, 2012)

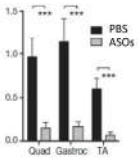
Gapmer-ASO treatment for DM1

CUG^{exp} ribonuclear foci reduction
(DM1 cell model)

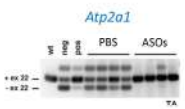


(Nakamori et al. *Mol Ther*, 2011)

Toxic RNA (CUG^{exp}) reduction
(DM1 model mice)



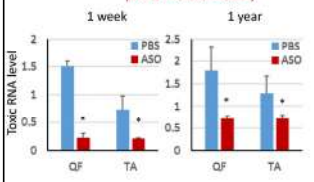
Splicing recovery
(DM1 model mice)



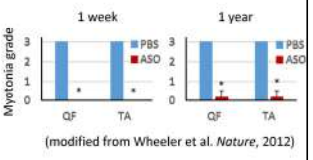
(Wheeler et al. *Nature*, 2012)

Gapmer-ASO treatment for DM1

Toxic RNA reduction
(DM1 mouse model)

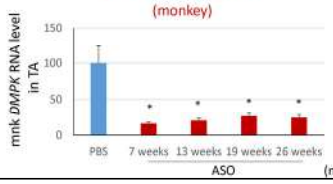


Improvement of myotonia
(DM1 mouse model)



(modified from Wheeler et al. *Nature*, 2012)

DMPK mRNA reduction
(monkey)



Phase 1/2a

(modified from Pandey et al. *JPET*, 2015)

Huntington Disease (HD)

Sustained Therapeutic Reversal of Huntington's Disease by Transient Repression of Huntingtin Synthesis

(*Neuron*, 2012)

Phase 1/2a

ALS

ALS1 (SOD1 mutation)

An antisense oligonucleotide against *SOD1* delivered intrathecally for patients with *SOD1* familial amyotrophic lateral sclerosis: a phase 1, randomised, first-in-man study

(*Lancet Neurol*, 2013)

Phase 1/2a

C9-ALS (G4C2 expansion)

Gain of Toxicity from ALS/FTD-Linked Repeat Expansions in C9ORF72 Is Alleviated by Antisense Oligonucleotides Targeting GGGGCC-Containing RNAs

(*Neuron*, 2016)

Familial Amyloid Polyneuropathy (FAP)

Clinical development of an antisense therapy for the treatment of transthyretin-associated polyneuropathy

(*Amyloid*, 2012)

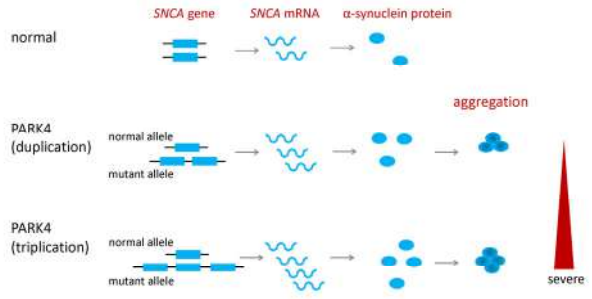
Phase 3

Parkinson's Disease (PD)

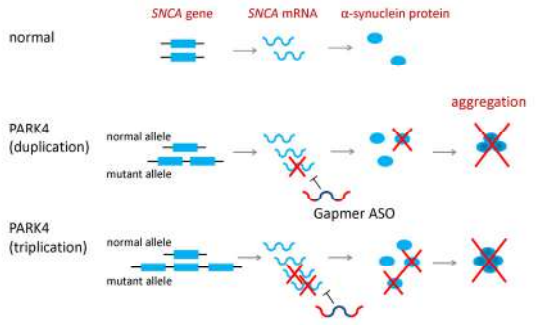
- A chronic progressive neurodegenerative movement disorder characterized by loss of nigrostriatal dopaminergic neurons with aggregated α-synuclein
- 2nd most common neurodegenerative disorder after Alzheimer disease
- Clinical features
  —tremor at rest, rigidity, akinesia (or bradykinesia), postural instability
- 5-10% of PD patients carry a mutation causing a monogenic form of the disorder

PARK4

- One form of hereditary Parkinson's disease (autosomal dominant)
- α-Synuclein (SNCA) duplication/triplication results in aggregation



ASO therapy for PARK4



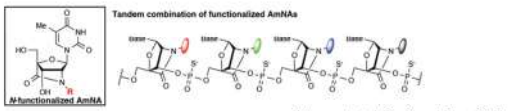
SNCA knockdown by oligonucleotides therapy

Publication	Oligonucleotides	Model
Kinoh et al. *BBRC*, 2006	AAV-ribozyme	Rat
Sapru et al. *Exp Neurol*, 2006	Lenti-shRNA	Rat
Gorbatyuk et al. *Mol Ther*, 2010	AAV-shRNA	Rat
Khodr et al. *Brain Res*, 2011	AAV-shRNA	Rat
Lewis et al. *Mol Neurodegener*, 2008	Naked siRNA	Mouse
Cooper et al. *Mov Disord*, 2014	Exosomal siRNA	Mouse
McCormack et al. *PLoS One*, 2010	siRNA (2-O-Me)	Monkey

Need non-viral, long-lasting, effective therapy

AmNA-ASO

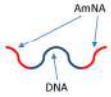
AmNA: Amido-bridged nucleic acids



(Yamamoto et al. *Org Biomol Chem*, 2015)

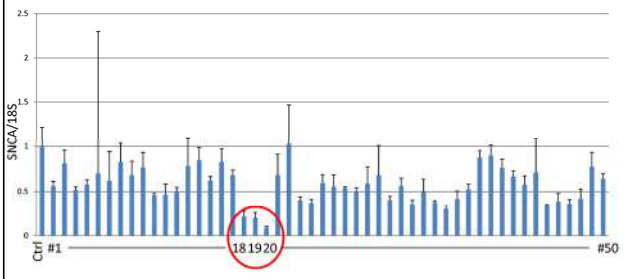
AmNA gapmer-ASO

- Excellent binding affinity to target RNA
- Improved nuclease resistance
- Low toxicity



Screening of AmNA-ASOs for α-synuclein (*SNCA*) knockdown

Efficacy of endogenous *SNCA* knockdown in HEK293 cells
(ASO @50nM)

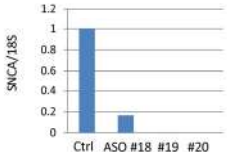


SNCA knockdown by AmNA-ASO in vivo

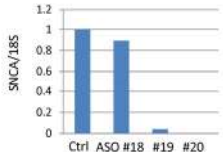
Human SNCA Tg mouse
(Wakamatsu et al., *Neurobiol Aging*, 2008)

AmNA-ASO (80 μg)
i.c.v. injection

Substantia nigra



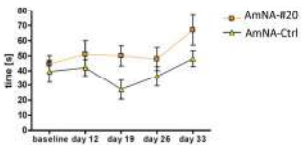
Striatum



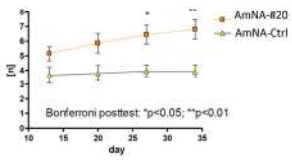
AmNA-ASO improves motor deficits in PD model mice

- Line61 PD model mouse
  human wildtype α-synuclein transgenic
  motor deficits at <2 months of age
- i.c.v injection of AmNA-ASOs (100 μg)

Wire suspension



Pasta handling



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  - — Duchenne Muscular Dystrophy, Spinal Muscular Atrophy
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  - — Parkinson Disease
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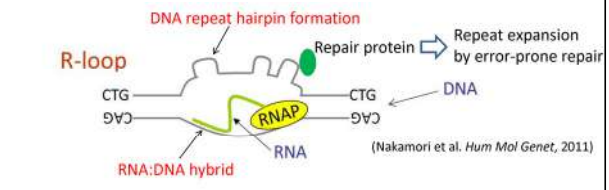
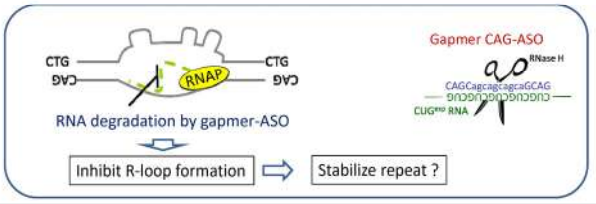
  - — Trinucleotide Repeat Expansion Disorders (TRED)

Trinucleotide repeat expansion disorders (TRED)

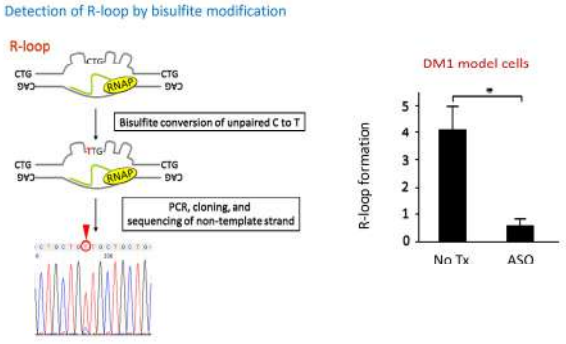
- A group of hereditary neurodegenerative disorders caused by expansion of triplet repeats
  CAG/CTG: Huntington Disease, Spinocerebellar Ataxia, Myotonic Dystrophy
  CGG: Fragile X syndrome GAA: Friedreich Ataxia
- Size of the expanded repeat correlates with the severity of the disease.
- The expanded repeats are unstable and continue to grow throughout the life.
- Stabilization of the repeat can be a means to slow the progression.

Mechanism of repeat instability

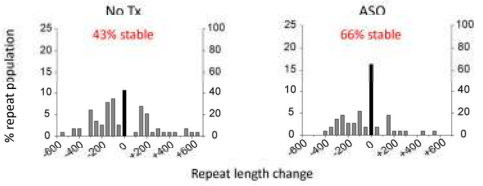




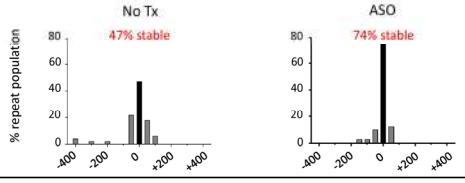
Gapmer CAG-ASO inhibits R-loop formation



CAG-ASOs stabilize an expanded CTG repeat in DM1 model cells



CAG-ASOs stabilize CTG repeats in DM1 model mice



Summary

- ASOs therapy for neurological/neuromuscular disorders
  - — high target specificity and affinity
  - — long duration of action
  - — relative ease of delivery to CNS or muscle
- Many clinical trials are ongoing
  - — HD, ALS, FAP, DM1 (target degradation)
  - — SMA, DMD (splicing modulation)
- Dual benefits for Trinucleotide Repeat Expansion Disorders
  - — reducing target RNA
  - — stabilizing repeats (slowing disease progression)

Acknowledgement

Osaka Univ.
Takuya Uehara
Chi-Jing Choong
Hideki Hayakawa
Hideki Mochizuki
Satoshi Obika
Kazuhiko Nakatani

Tokyo Medical & Dental Univ.
Tetsuya Nagata
Takanori Yokota

Univ. of Rochester
Charles Thornton

Hospital for Sick Children
Christopher Pearson

Univ. of Florida
Andy Berglund

Université Paris Descartes
Geneviève Gourdon