

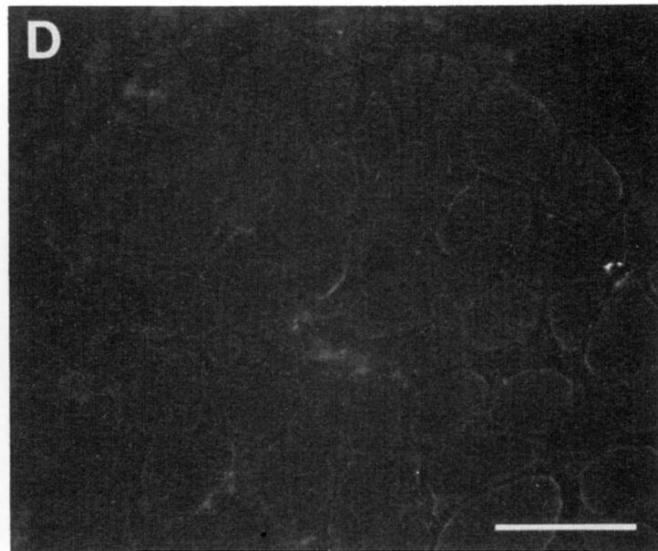
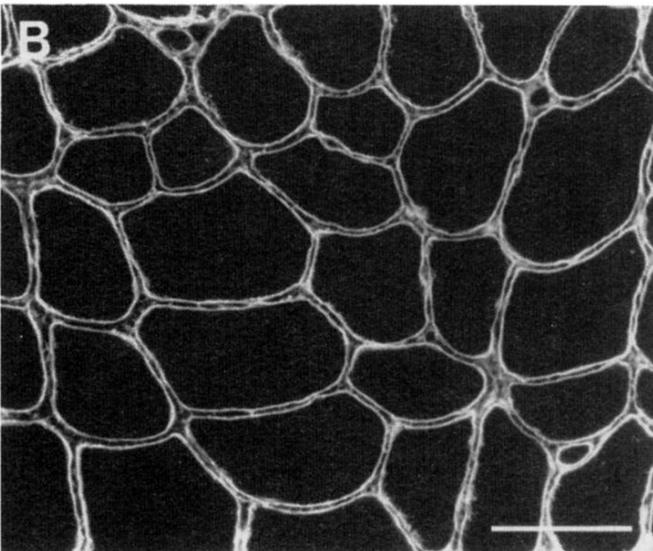
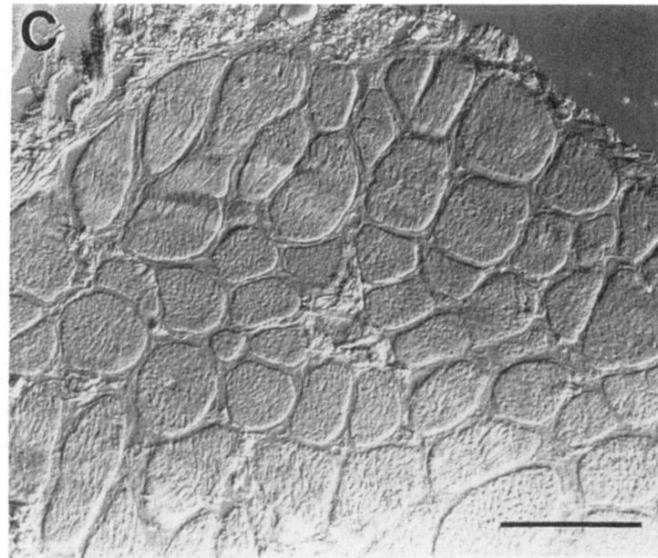
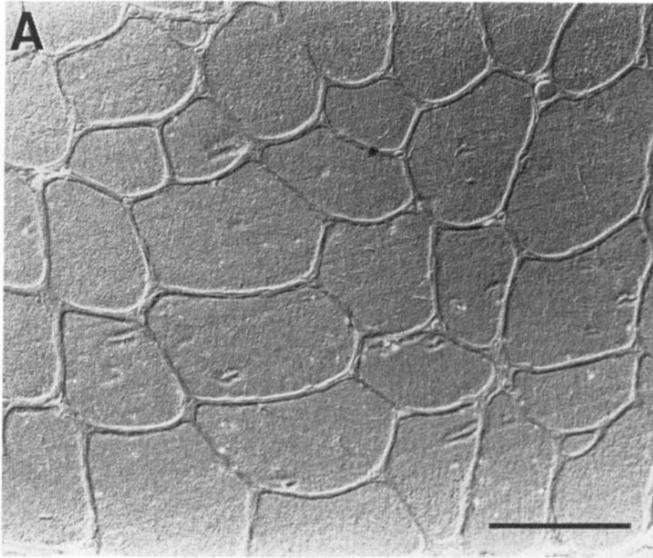
Duchenne muscular dystrophy



Duchenne muscular dystrophy:

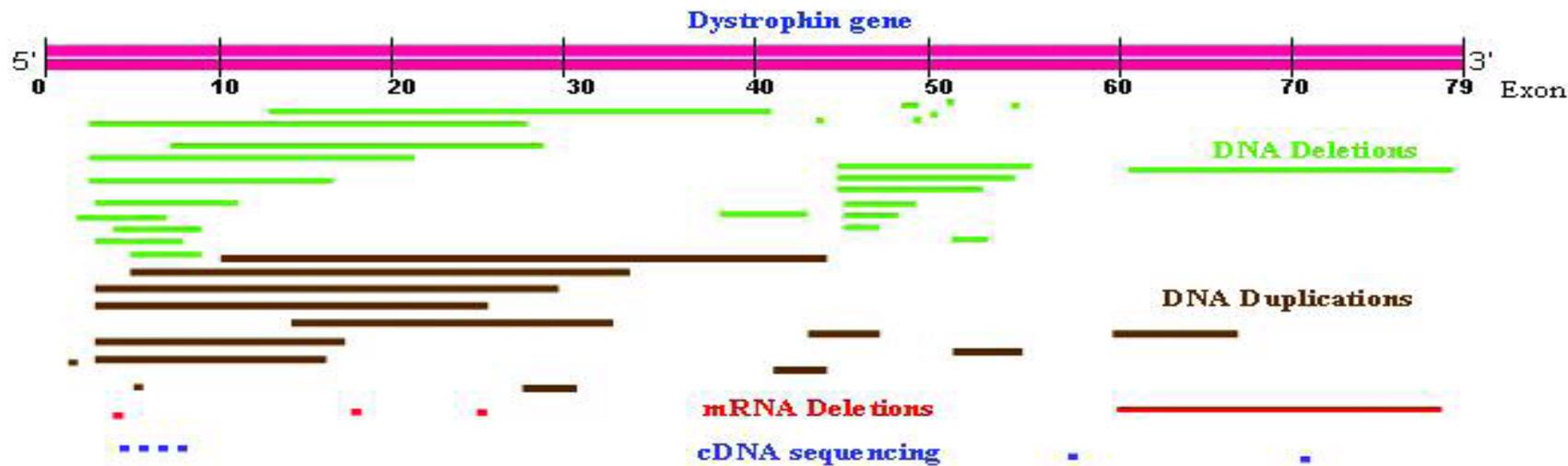
Clinical features

- X linked recessive – mostly males
- High serum creatine kinase from birth – neonatal screening possible
- Presentation early school years – difficulty keeping up with peers
- Progressive muscle weakness and loss
- Loss of ambulation ~10 yrs, loss of activities daily living 15 yrs - ventilation
- Daily glucocorticoids – increase in strength, delay in loss of ambulation
- Cardiac involvement - failure



Normal Dystrophin

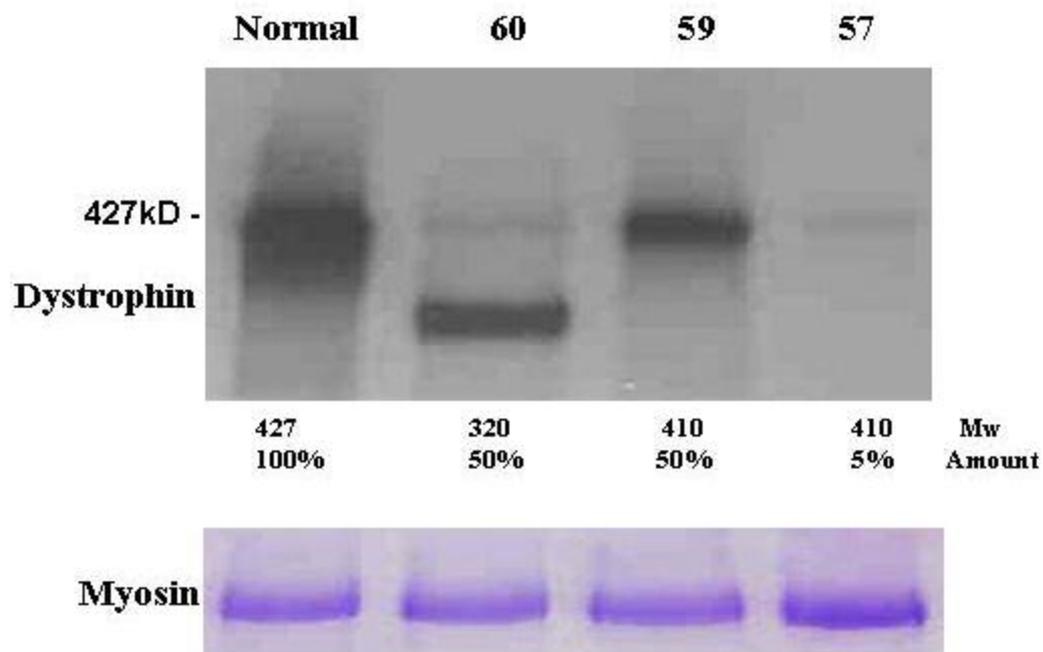
Duchenne muscular dystrophy

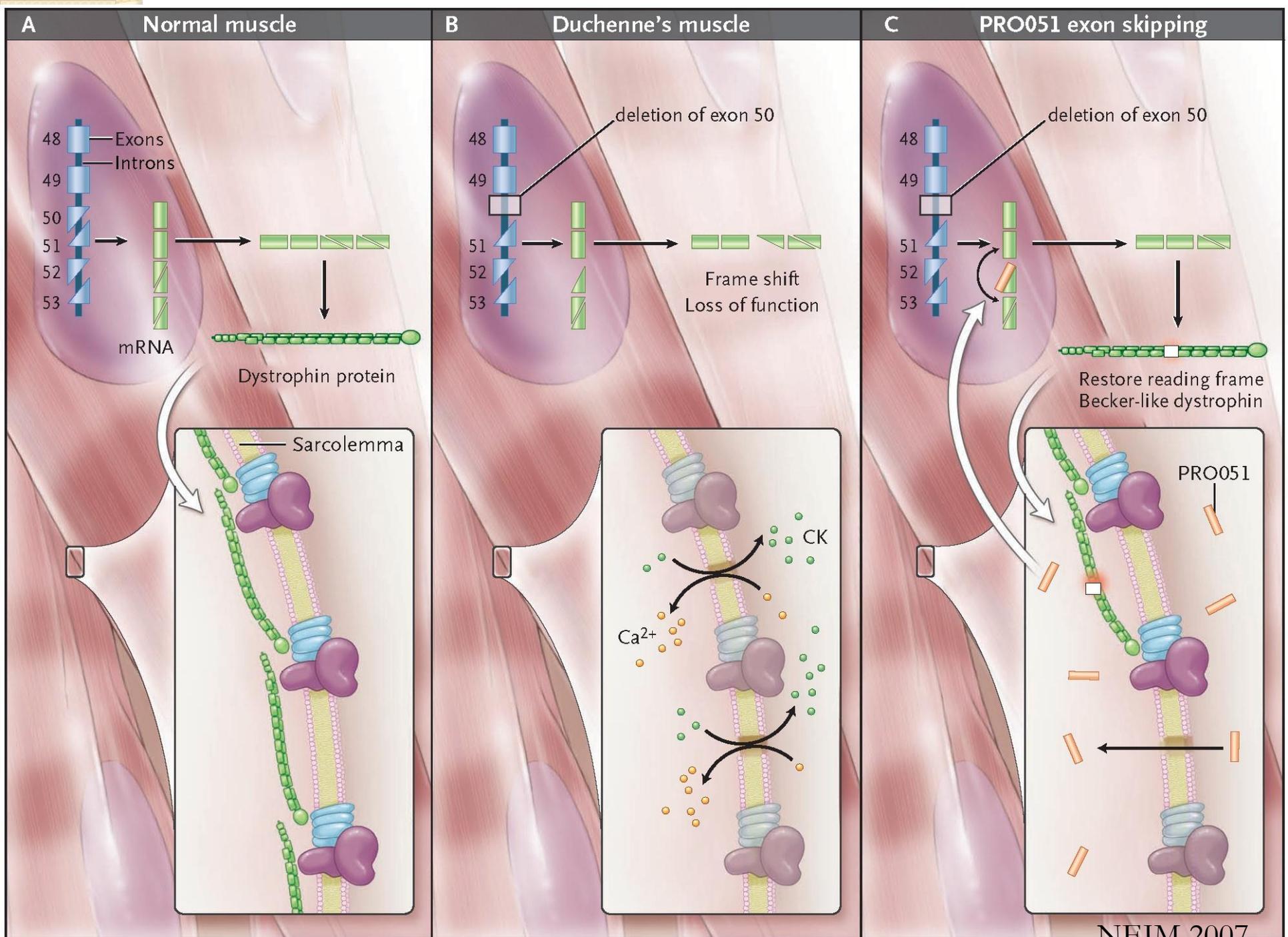


Duchenne dystrophy =
Absence of dystrophin
Complete loss of function

Becker dystrophy =
Present, but abnormal
Partial loss of function

Large in-frame deletions
Can be clinically very mild,
asymptomatic
(hyperCKemia)



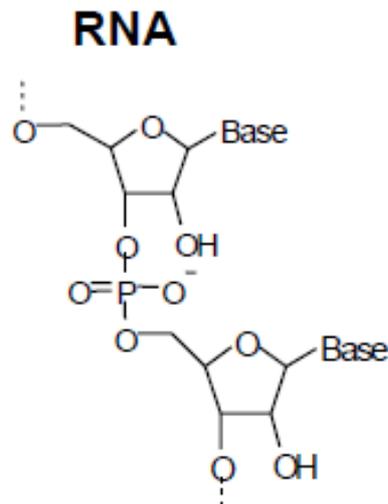
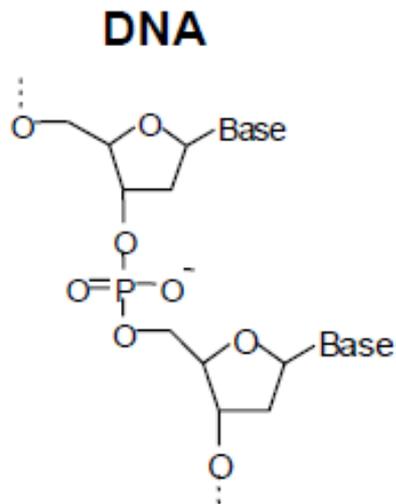


Clinical applications of anti-sense:

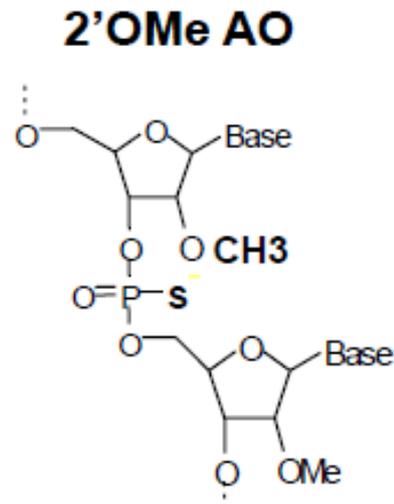
- 20 yrs; 90 clinical trials; 40 completed
- >2,000 patients, targeting cancer, inflammatory disease, and other indications.
- A single AO has been FDA approved
 - Vitravene®, intraocular injection to inhibit cytomegalovirus retinitis (CMV) in immunocompromised patients; Isis Pharmaceuticals
 - No longer marketed.
- **Anti-sense barriers**
 - Sufficient intracellular drug for biochemical efficacy
 - Therapeutic window

Systemic anti-sense in Duchenne

- **~100-fold increase in target efficacy**
 - Drug entry to cells facilitated by overt breaches in myofiber cell membranes (bulk flow into cell)
 - Previous knock-downs: goal **90%** of mRNA targets
 - Dystrophin mRNA rescue: **10%** of mRNA targets

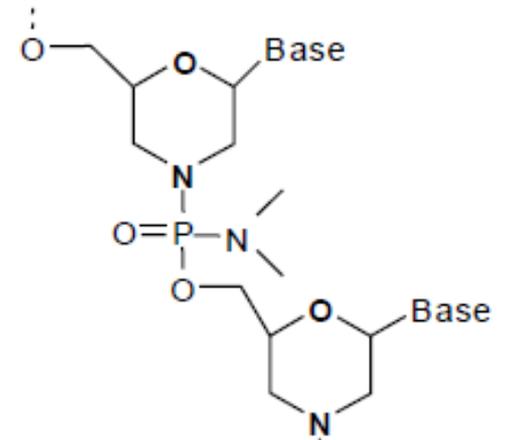


Prosensa/GSK



AVI Biopharma

Morpholinos (PMO)

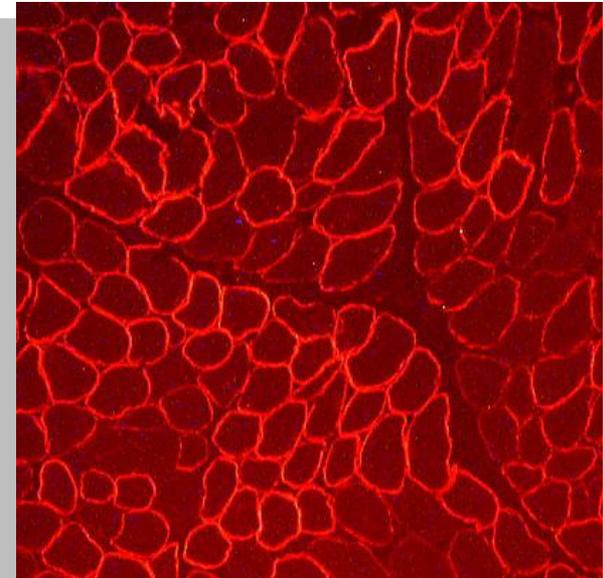
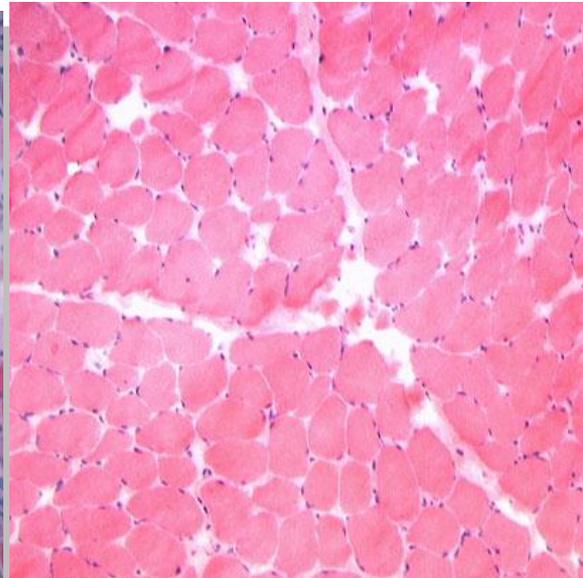
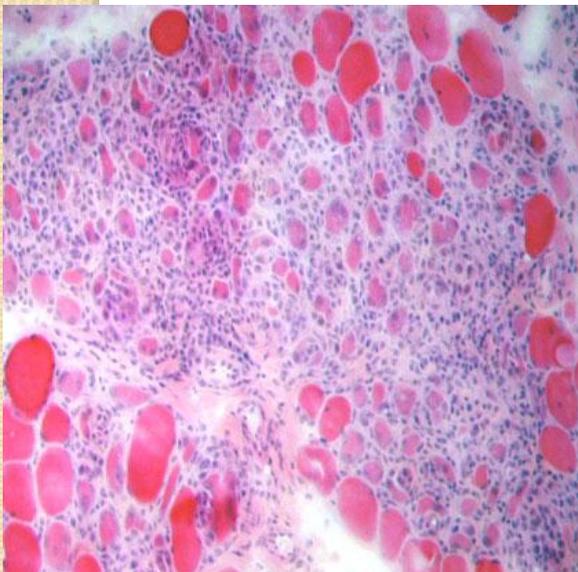


Proof of principle: Large animal dog model

- Spontaneous LOF mutations in dogs
- Similar to human disease
 - Progressive weakness, death by 6 months
- Challenging mutation
 - Splicing near amino-terminus;
 - Required targeted 2 exons
- Tested drug combinations by intramuscular injection

Non-treated littermate

3 morpholino treated CXMD



Systemic Delivery



40-60 mg/kg@AO x 3 AOs

Weekly IV dose =
120-200 mg/kg

Cumulative dose =
1.4 grams/dog
2-4 months treatment

Endpoints:

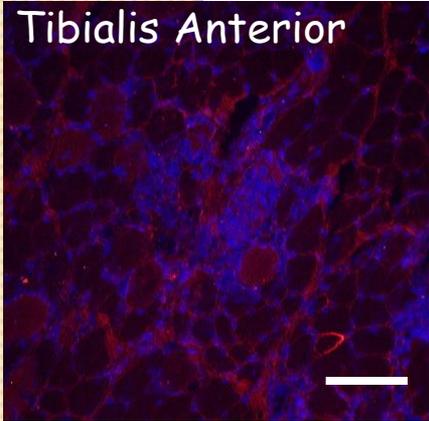
- 1 Dystrophin by blot, Immunostaining
- 2 Histology, Functional testing, Symptom grading, MRI
- 3 Toxicology

Yokota et al. Annals Neurology 2009

Recovery of dystrophin expression after systemic morpholino treatment in CXMD

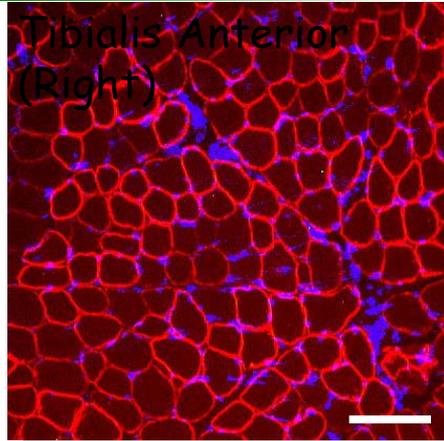
CXMD non-treated

Tibialis Anterior

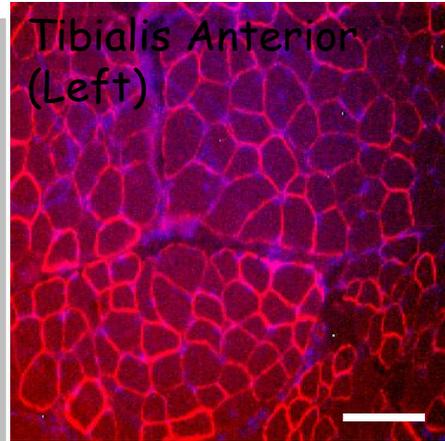


Cocktail morpholinos (5 inj x 120 mg/Kg in total) treated CXMD

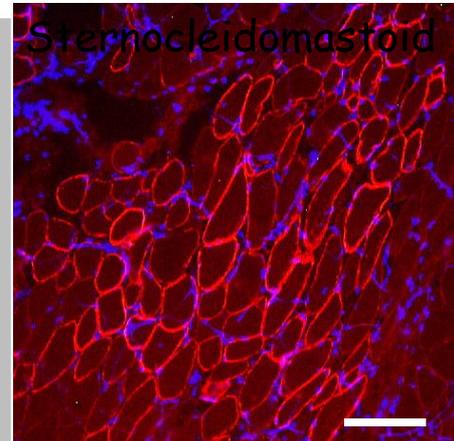
Tibialis Anterior (Right)



Tibialis Anterior (Left)

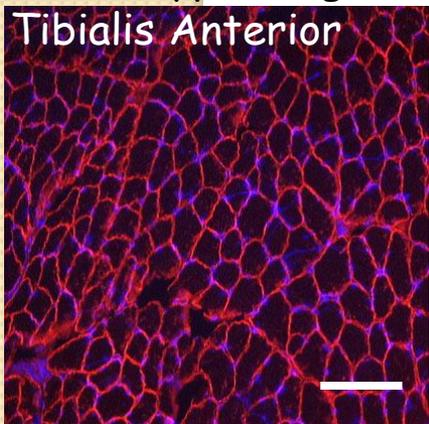


Sternocleidomastoid

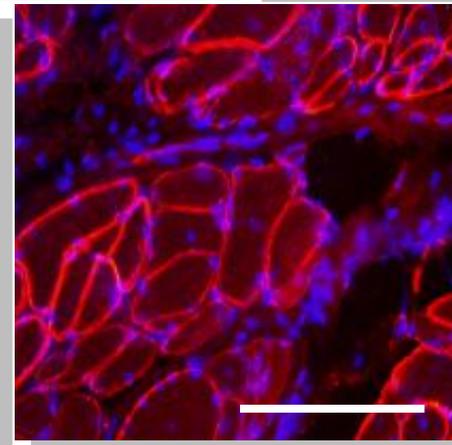
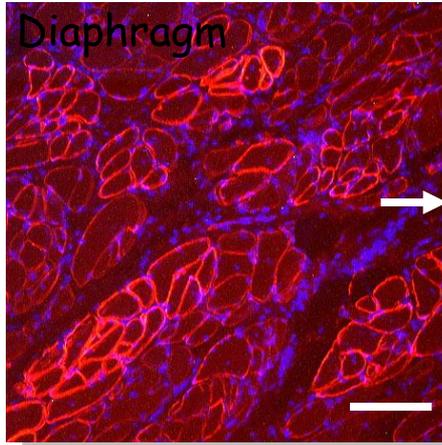


Wild-type beagle

Tibialis Anterior



Diaphragm



Dystrophin (Dys-1) and nuclear staining at 15 days after 5 x injections with 6 g of morpholinos in total targeting exon 6 and 8 (cocktail of Ex6A, Ex6B, Ex8A) into young adult CXMD. Bars; 100 μ m

Dogs and Morpholinos: Dystrophin rescue: Variable, average ~20%

Wild-type Tibialis Anterior
(1/2 Dilution)

Wild-type (1/10 Dilution)

CXMD non-treated (TA)

Triceps Brachii

Biceps Brachii

Diaphragm

Esophagus

Tibialis Anterior

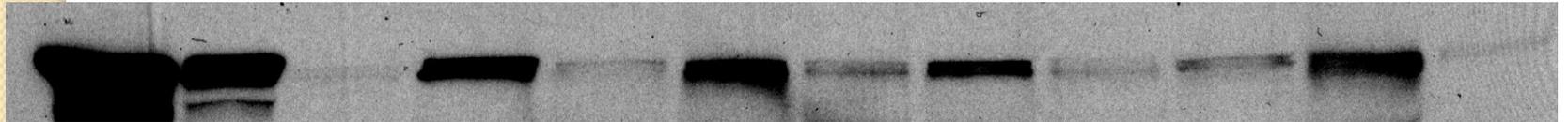
Adductor magnus

Extensor digitorum longus

Masseter

Heart

5 x 120 mg/Kg Morpholino treated



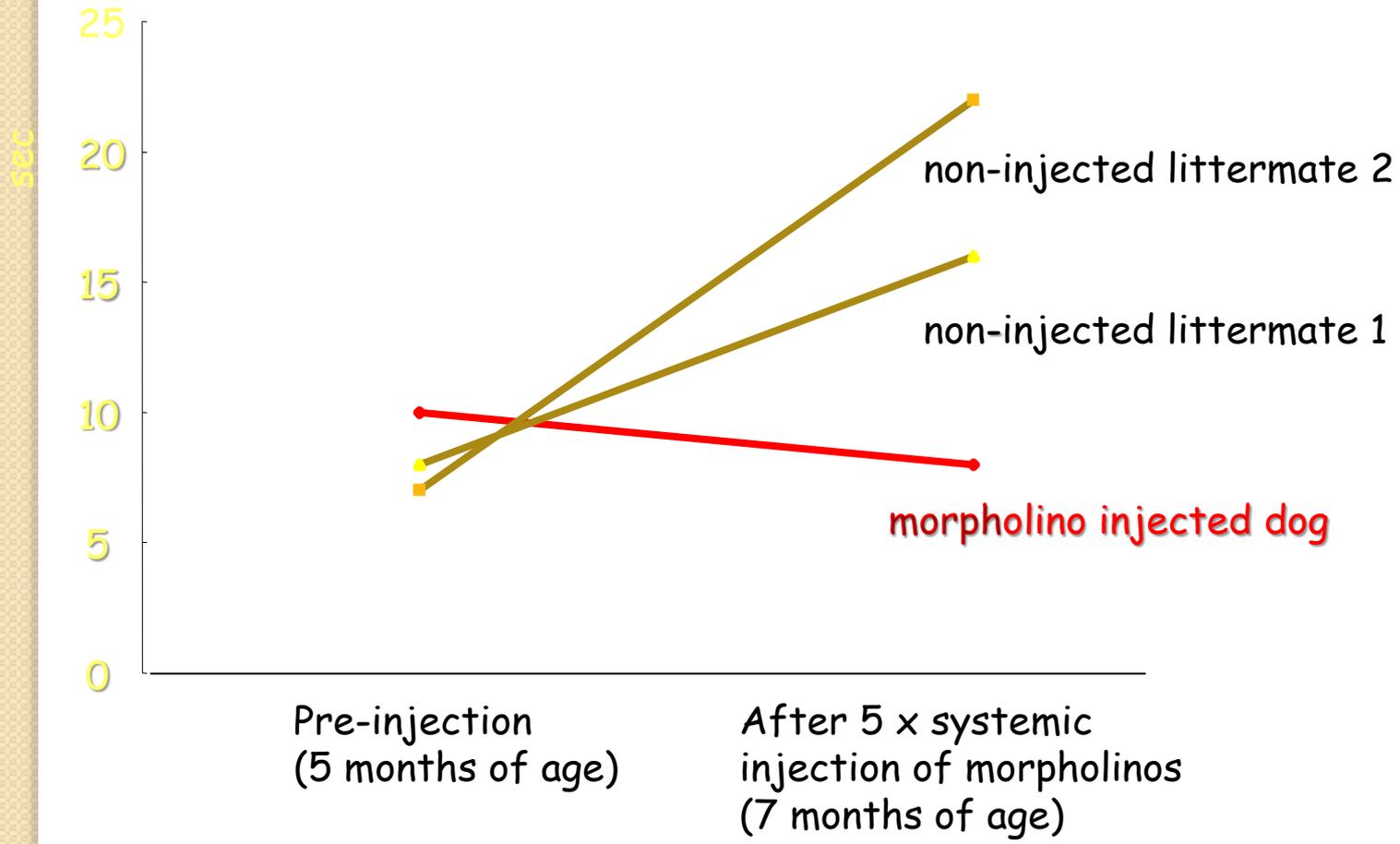
DYS1 (dystrophin)



Desmin

50 μ g, 10 μ g, or 100 μ g of total proteins were loaded in each lane as indicated.

15 m Running test before and after morpholino injection



Running test of littermates



Non-treated
littermate



11 x weekly
treated littermate

GLP Tox

- Normal animals/humans
 - Will drugs skip normal gene, induce DMD?
- EMEA: Do tox in DMD patients
- FDA – more tox studies required (AVI; DoD; FED; CureDuchenne)
 - Human DMD drug (AVI 4658)
 - Normal mice – 12 wk weekly IV dose
 - 960 mg/kg/wk
 - Non-human primates – 12 wk weekly IV dose
 - 320 mg/kg
 - Mouse mdx drug (AVI 4225)
 - Mdx mice – 12 wk weekly dosing up to
 - 960 mg/kg

AVI-4658 and AVI-4225 Mouse Study Summary

- No dose related changes in urine or serum kidney parameters
- No drug related clinical chemistry findings in all groups
- No test article-related effects on urinalysis parameters
- No adverse clinical observations
- **AVI-4225 in mdx mice:** Microscopic evaluation shows improvement in myofiber degeneration as a result of treatment with AVI-4225 in mdx mice

Dose level: mg/kg (<i>mdx</i> strain)	0		12		120		960		960	
	(IV)		(IV)		(IV)		(IV)		(SC)	
Sex	M	F	M	F	M	F	M	F	M	F
Biceps femoris	10	10	6	9	4	8	5	5	7	7
-minimal	2	0	5	4	4	4	5	5	5	3
-mild	5	6	1	5	0	4	0	0	2	4
-moderate	3	4	0	0	0	0	0	0	0	0

IV

SubQ

Less effective

GLP Tox: Morpholinos

- Excellent therapeutic window
 - Efficacy ~40 mg/kg
 - GLP Primates 320 mg/kg; Mice 960 mg/kg
 - Morpholinos not metabolized
 - Traditional dose equivalencies: permits dosing humans to 100 mg/kg
- Do not induce DMD in normal muscle
- Consistent, predictable pharmacokinetics
- Subcutaneous less effective than IV

Status of clinical development programs: *completed studies*

- Morpholinos (AVI)
 - Open label dose escalation study
 - Francesco Muntoni, UCL
 - [Lancet](#). 2011 Aug 13;378:595-605.
 - 19 patients, 0.5 – 20 mg/kg/wk IV, 12 wks
 - Convincing dystrophin expression in muscle; few patients at potentially therapeutic levels
- 2'Omethyl (Prosensa/GSK)
 - Open label dose escalation study
 - Nathalie Goemens, Leuven
 - [N Engl J Med](#). 2011 Apr 21;364:1513-22.
 - 12 patients, 0.5 – 6 mg/kg/wk subcutaneous, 5 wks
 - + 12 wk extension at 6 mg/kg/wk
 - Some dystrophin expression

Ongoing 2'Omethyl Clinical Studies

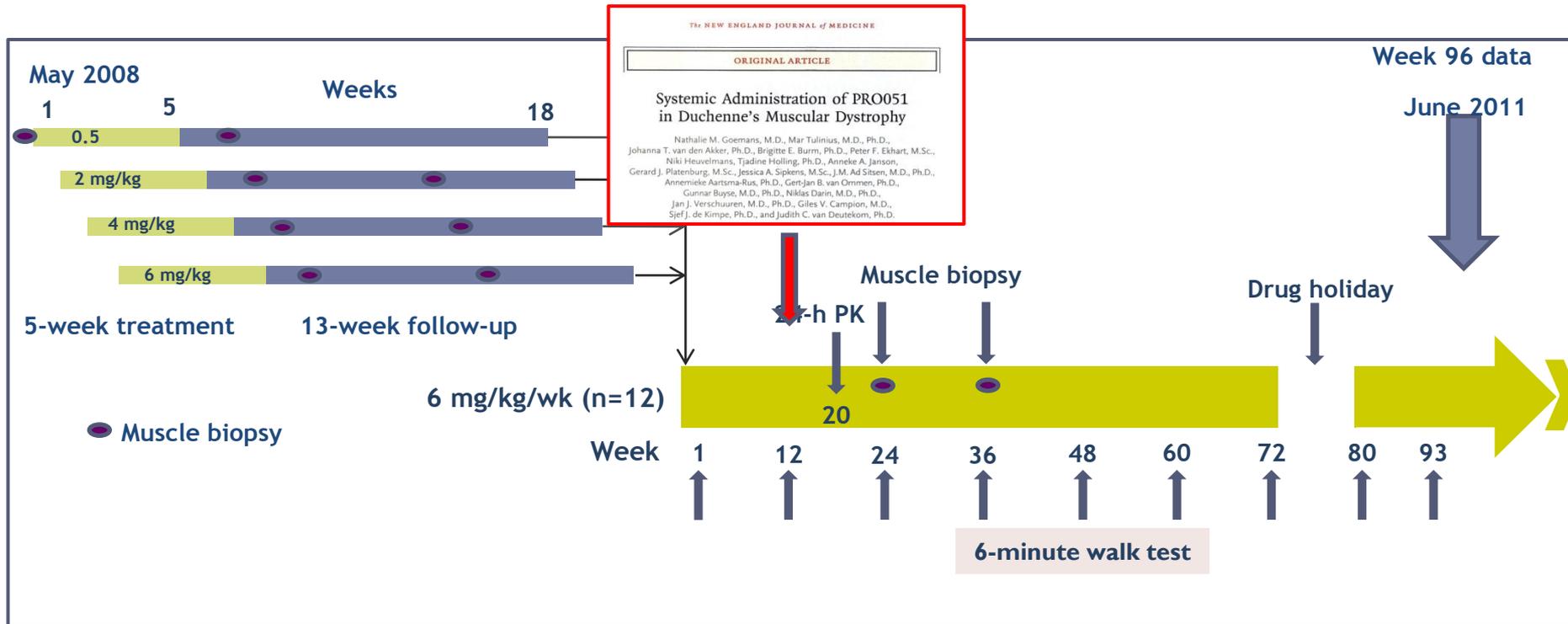
- **Study DMDI 14117 (regime optimization, EU, Australia, Turkey, Israel)**
 - Ambulant, double blind placebo-controlled, two dosing regimes vs placebo, 12 sites
- **Study DMDI 14118 (single dose PK, USA + France)**
 - Non-ambulant, single dose, dose-escalating tolerability and PK, 2 sites
- **Study DMDI 14044 (pivotal, global excl. USA)**
 - Double-blind, placebo-controlled, 6mg/kg vs placebo, 35 sites
- **DMDI 14876 (USA)**
 - 2 different doses of SC GSK2402968 versus placebo administered over 24 weeks in ambulant subjects with DMD.

Ongoing Morpholino Clinical Studies

- **Two dose, blinded, placebo-controlled (dose-finding, USA)**
 - 30 mg/kg; 50 mg/kg wk IV; 12 patients; 12 wk with extension study

Completed Phase I/IIa open label dose escalation study + 12 week extension reported

N Engl J Med. 2011;364:1513-22.



Endpoints

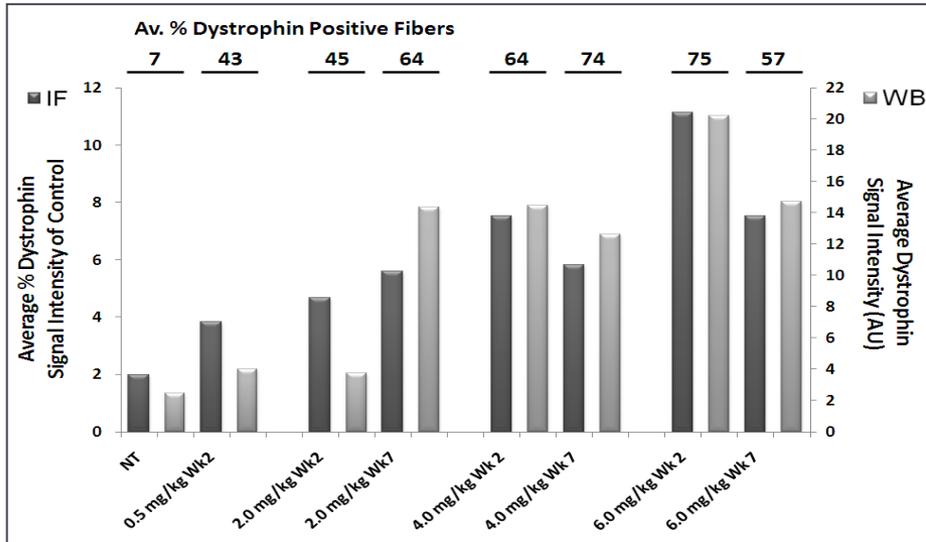
- Safety and tolerability
- Plasma and tissue pharmacokinetics
- Muscle biopsies: RNA and protein effects
- Muscle strength and function

Safety and efficacy assessments

- Weekly: AEs, urinalysis (Weeks 1–16)
- 2-weekly: thrombocytes, urinalysis (Weeks 16–96)
- Monthly: safety, blood and urine, PK (to Week 24), ECGs, muscle strength and function (Weeks 8–96)

Completed Phase I/IIa open label dose escalation study + 12 week extension reported

N Engl J Med. 2011;364:1513-22

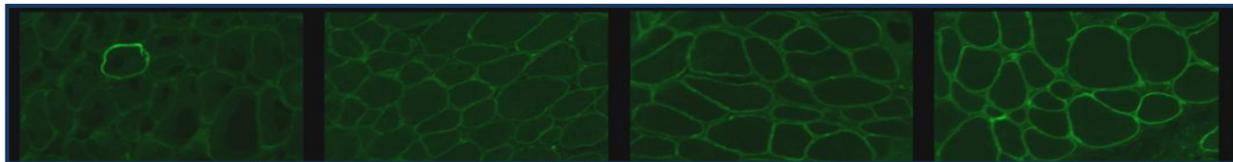


Summary:

- First successful systemic administration of GSK2402968
- Favorable pharmacokinetic profile
- Dose dependent increase in dystrophin expression
- Well tolerated at 6 mg/kg sc (12w extension)

Increasing dose →

Pre-Treatment

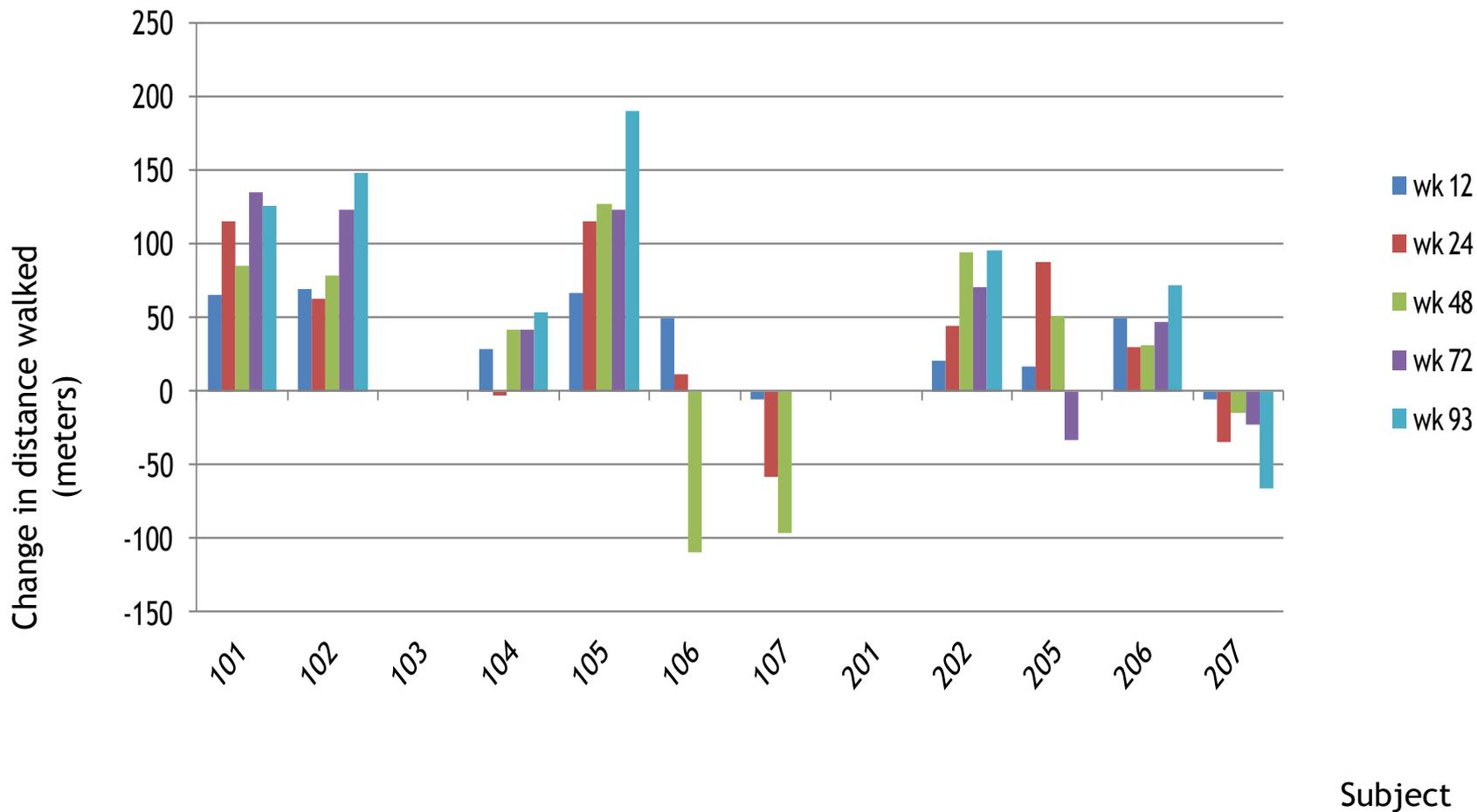


Dystrophin (ManDys 106)

Selected dose:
6 mg/kg

Tissue levels of
GSK2402968 in
muscle biopsy at this
dose:
6.9 ± 1.9 µg/g

6-Minute Walk Test: 93-Week Extension at 6 mg/kg/week



N=10 (subjects who completed all 6-minute walk test [6MWT] assessments). Subject 103 stopped test early and is not included in Figure; subject 201 was non-ambulant at baseline. Subjects 106 and 107 not able to attempt 6MWT at 93 weeks - still included in mean change.

Summary

- ▶ GSK2402968 was generally well tolerated after 96 weeks
- ◀ Renal effects, thrombocytes and local injection-site reactions warrant continued monitoring
 - ▶ Reversibility of renal effects during off-treatment period was observed after intermittent dosing
- ▶ Considering the expected disease progression, encouraging results in 6-minute walk distance were observed in 7 out of 10 ambulant boys (P4.27)
- ▶ Larger placebo-controlled studies (DMD114117 and DMD114044) are currently ongoing

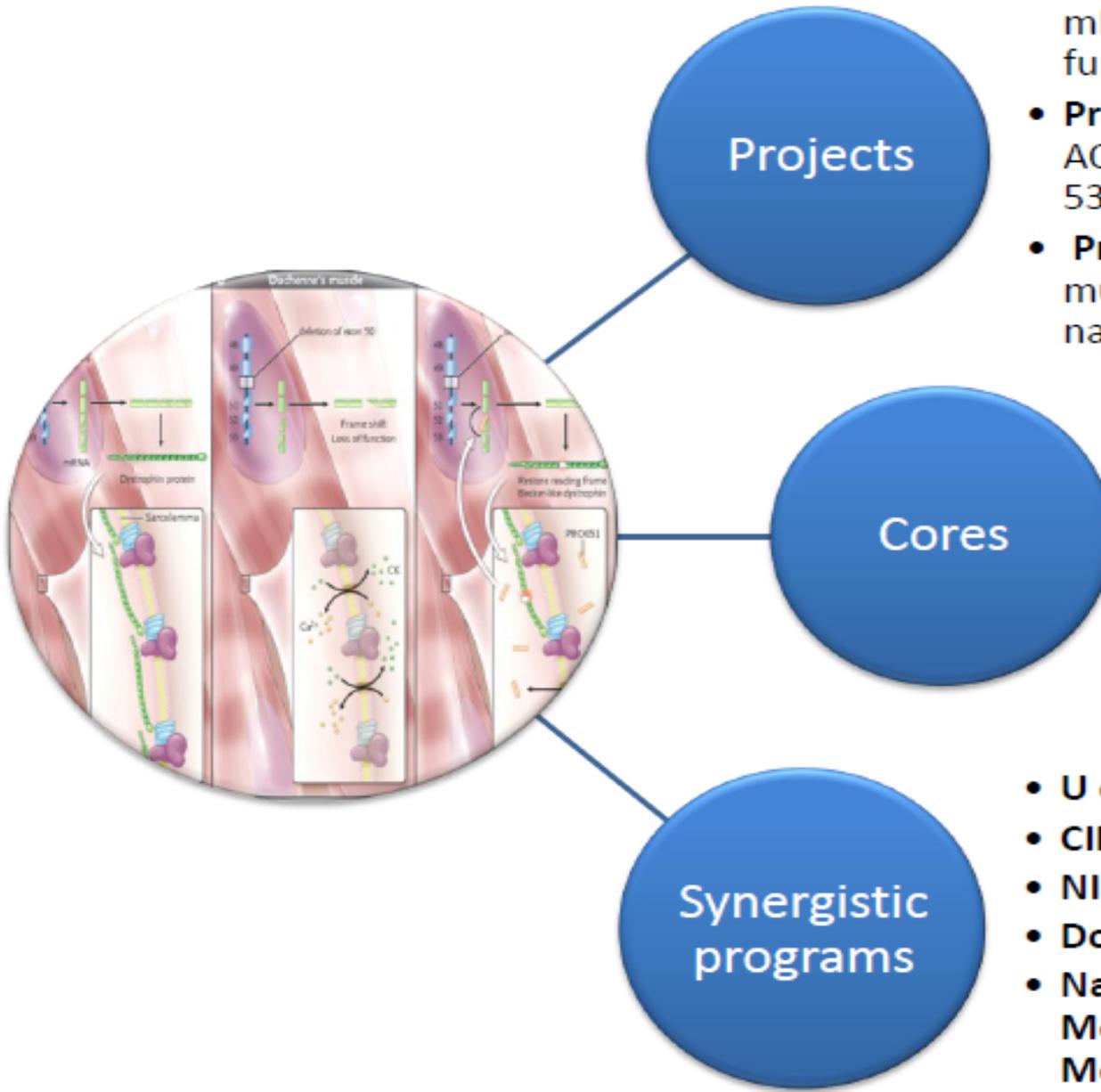


Challenges and unknowns

- Many exonic targets.
- BMD-like dystrophin function.
- Pre-clinical efficacy studies.
- AO target sequence selection.
- Long-term chronic tox.

- Goal:
 - Multiple exons – reduced regulatory hurdles
 - Coordination of international research community

NIAMS P50: Center of Research Translation on Exon Skipping (Hoffman, Clemens)



- **Project 1:** Dystrophin mRNA fidelity and protein function.
- **Project 2:** Optimization of AO drugs to exons 45, 51, 53.
- **Project 3:** Becker muscular dystrophy natural history.

- **Core A:** Administrative.
- **Core B:** In vitro and in vivo functional assays.
- **Core C:** Molecular diagnostics and tissue banking.

- U of Pitt CTSA; CNMC CTSA
- CINRG network
- NIH U54 ex45
- DoD Program Project
- National Center for Medical Rehabilitation Medicine

NICHD U54: Pediatric pharmacology center at Children's National Medical Center

Pediatric toxicity and efficacy in long-term systemic treatment with anti-sense: A case study of personalized medicine.

John Van den Anker, Edward Connor

NICHD Steering Committee

External Advisory Committee

Project 1. Clinical evaluation of urine biomarkers for morpholino accumulation and resolution in renal epithelial cells.

John Van den Anker,
Edward Connor, Jerry
Mendell

Project 2. Project 2. Biomarker discovery for AO accumulation in kidney.

Eric Hoffman
Yetrib Hathout

Project 3. Preclinical dosing optimization: Dosing schedule, tissue bioavailability, and functional outcome measures.

Kanneboyina Nagaraju,
Qi Lu

Core B. Bioanalytical Core

Pedro Jose, Robin A. Felder, Kristy Brown, Patricio Soares da Silva

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