LEIDEN UNIVERSITY MEDICAL CENTER

Development of antisense-mediated exon skipping for DMD Retrospective view & Lessons learned

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June 1 2015

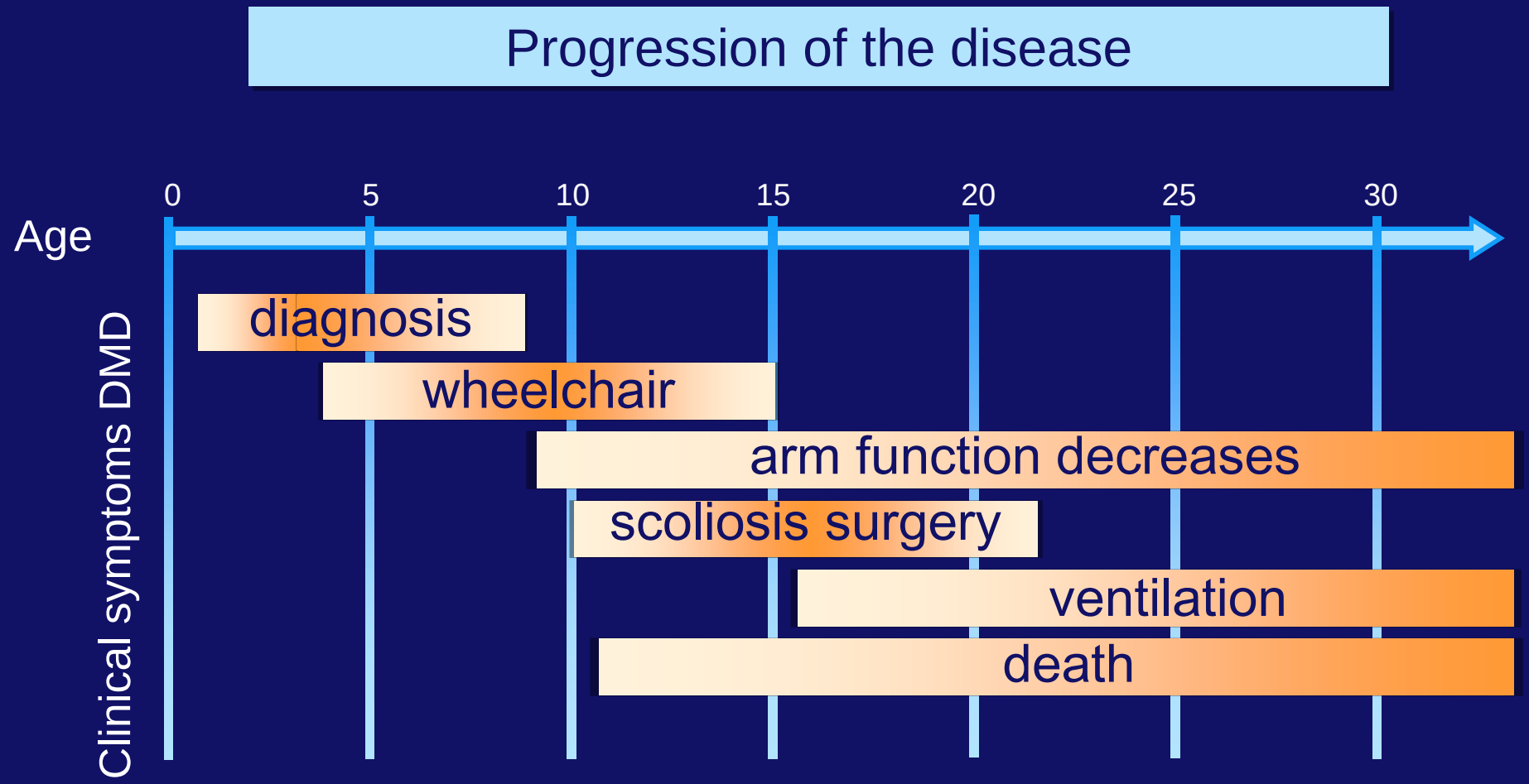
Towards a marketed drug

- Fundamental research
- Proof of concept studies
- Preclinical studies
- Clinical studies
- Marketing authorization (regulators, EMA)
- Health insurance/Implementation
- (Post marketing studies/MEB)

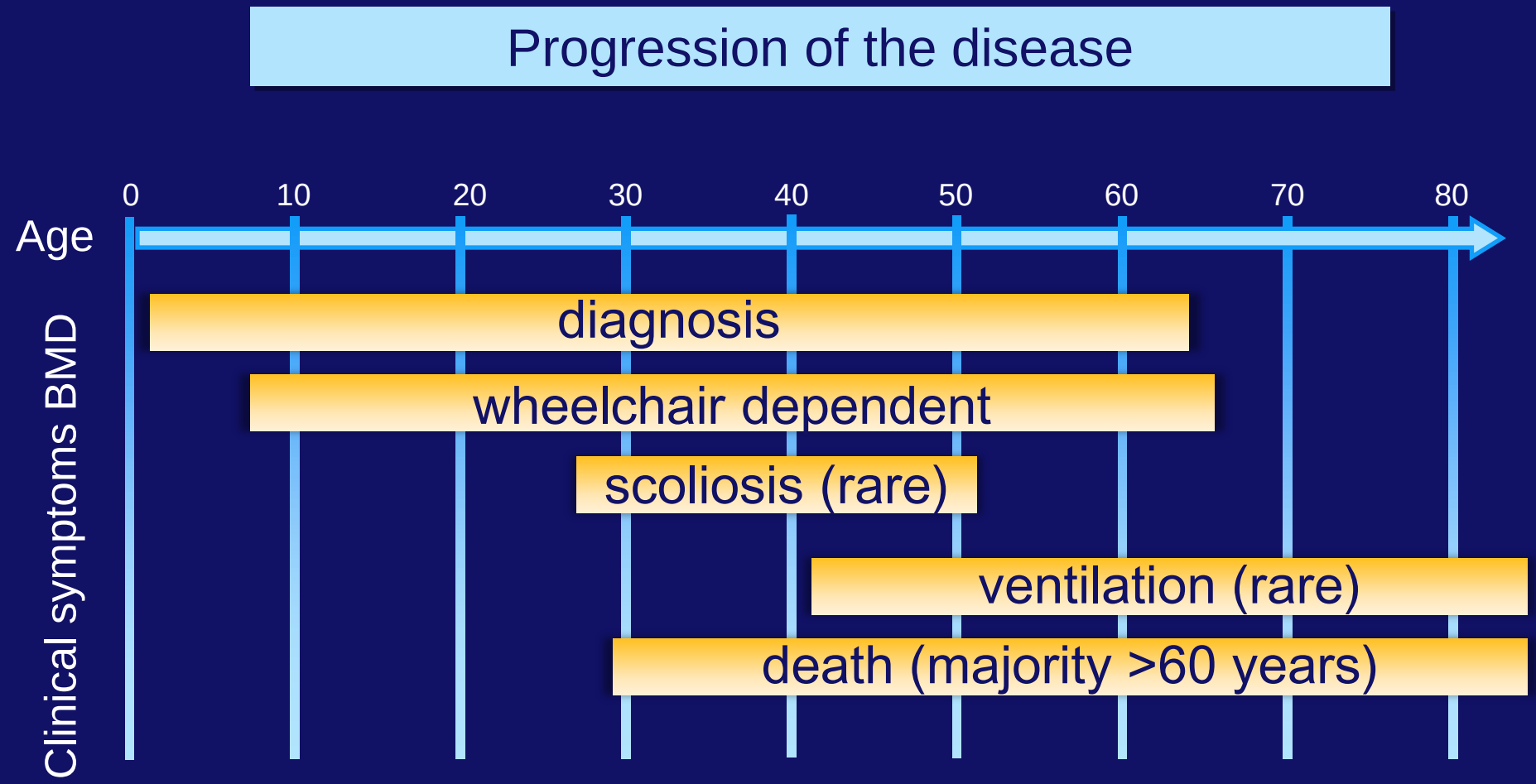
How are drugs approved?

- In Europe drugs for rare diseases are approved by European Medicine Agency (EMA)
- Regulators approve drugs based on a positive benefit/risk analysis
- ‘Clinical benefit’ important
- Conditional approval vs full approval
- Once a drug is approved, the company then has to market it in each European country

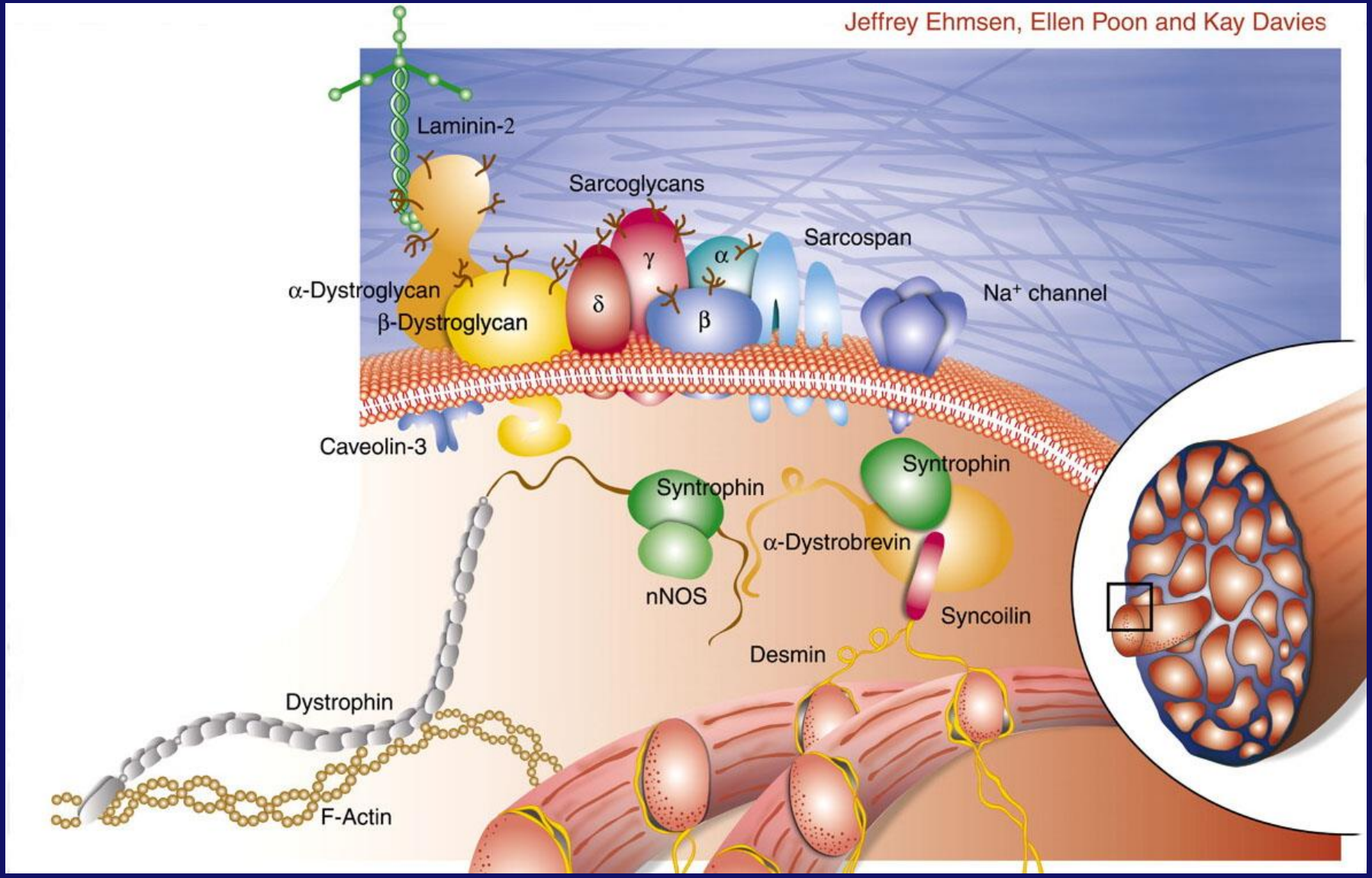
Duchenne Muscular Dystrophy



Becker Muscular Dystrophy (BMD)

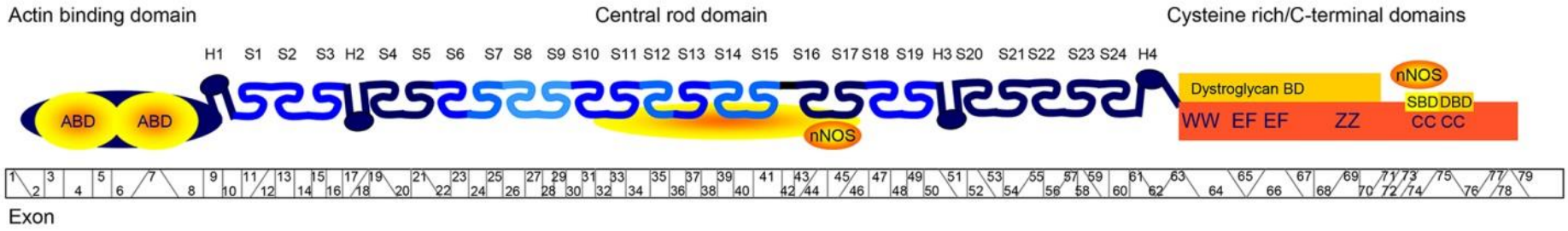


Dystrophin


Jeffrey Ehmsen, Ellen Poon and Kay Davies
Laminin-2
Sarcoglycans
Sarcospan
α-Dystroglycan
β-Dystroglycan
γ
α
δ
β
Na⁺ channel
Caveolin-3
Syntrophin
Syntrophin
α-Dystrobrevin
nNOS
Syncoilin
Desmin
Dystrophin
F-Actin

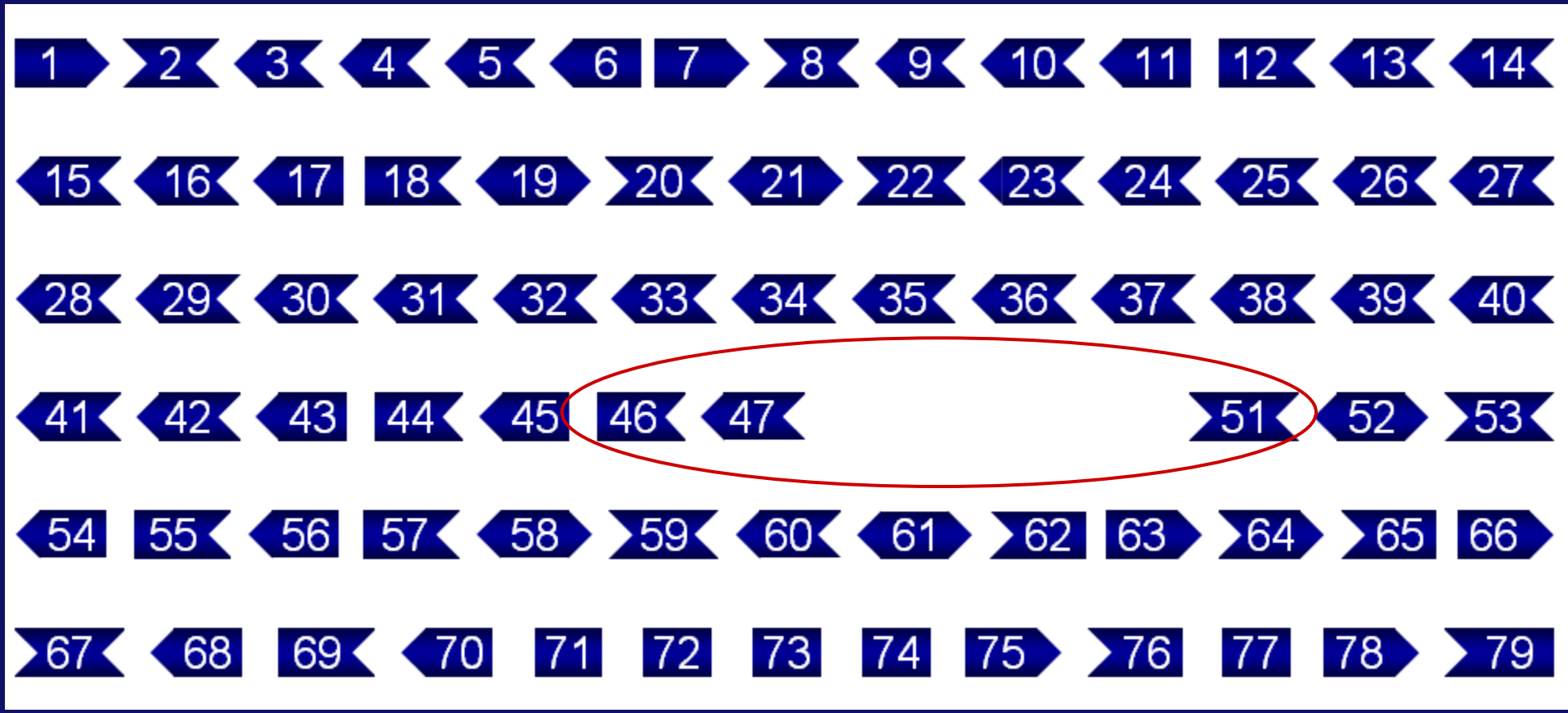
Dystrophin

- Acts as shock absorber
- Connects cytoskelletal actin to extracellular matrix



- Duchenne: Dystrophin prematurely truncated
  Not functional
- Becker: Dystrophin internally deleted
  Partially functional

Duchenne: reading frame disrupted



Exon 48-50 deletion

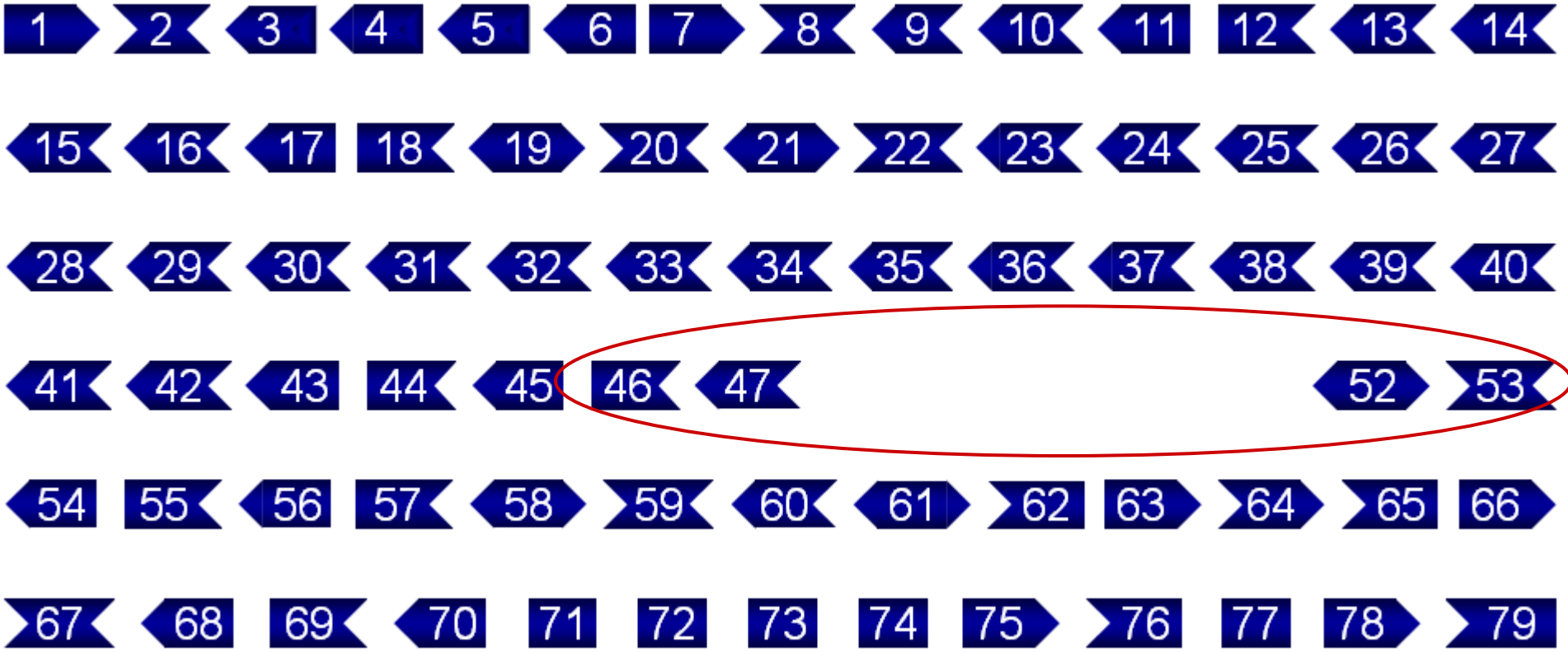
Reading frame disrupted

↓

Protein translation stops prematurely

Dystrophin not functional

Becker: reading frame maintained

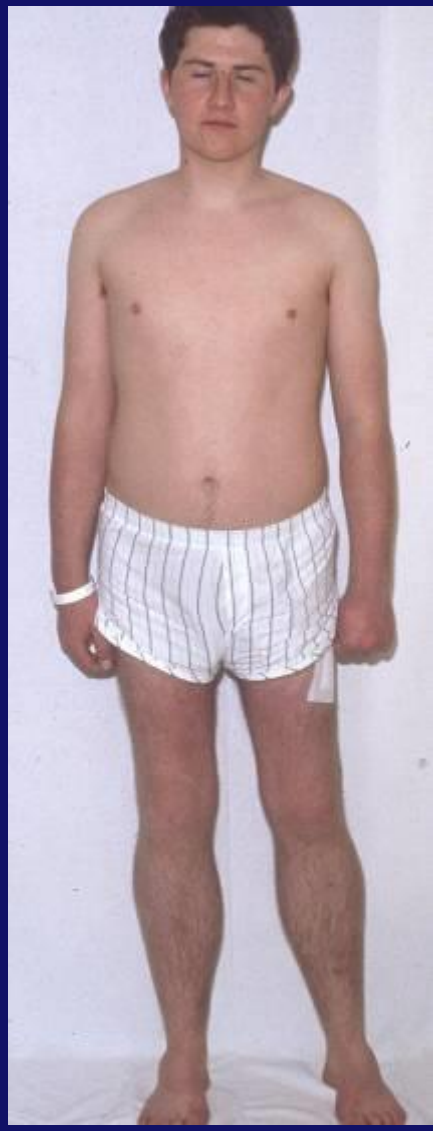


Becker: reading frame maintained

Protein translation continues

Dystrophin partly functional

Duchenne vs Becker



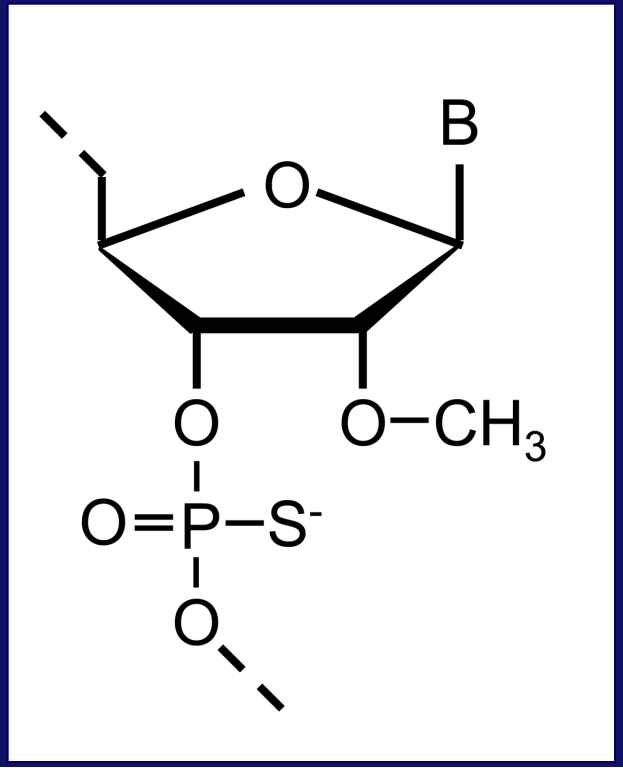
Exon 51 skipping

Reading frame restored

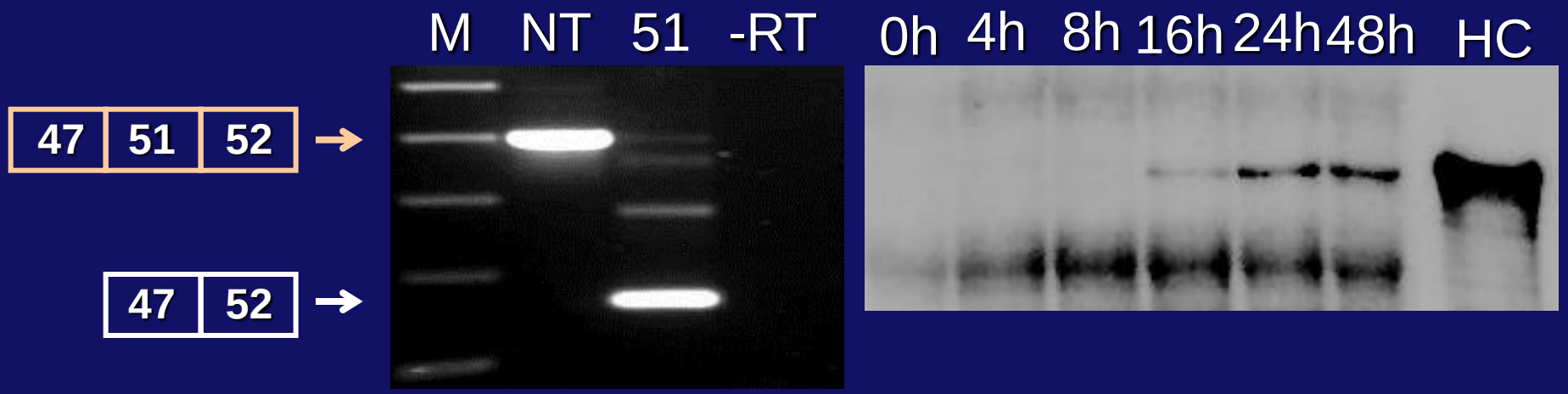
Partially functional dystrophin

AONs

- Modified pieces of RNA/DNA
- Many different modifications possible
- We use 2’-O-methyl phosphorothioate
- Transfection needed in cell culture
- Saline injection possible in mouse

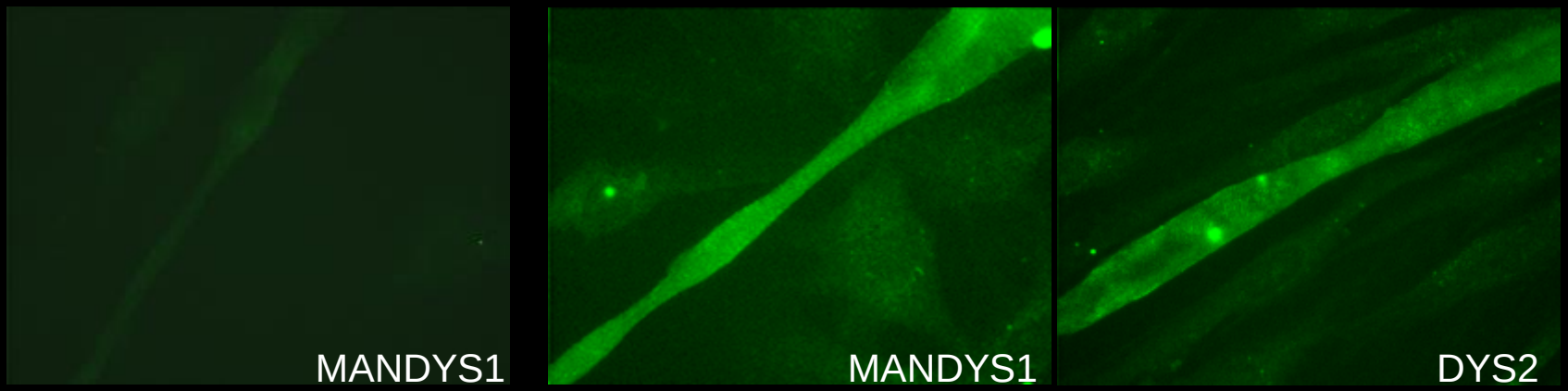


Exon 51 skipping in Δ exon 48-50 cells

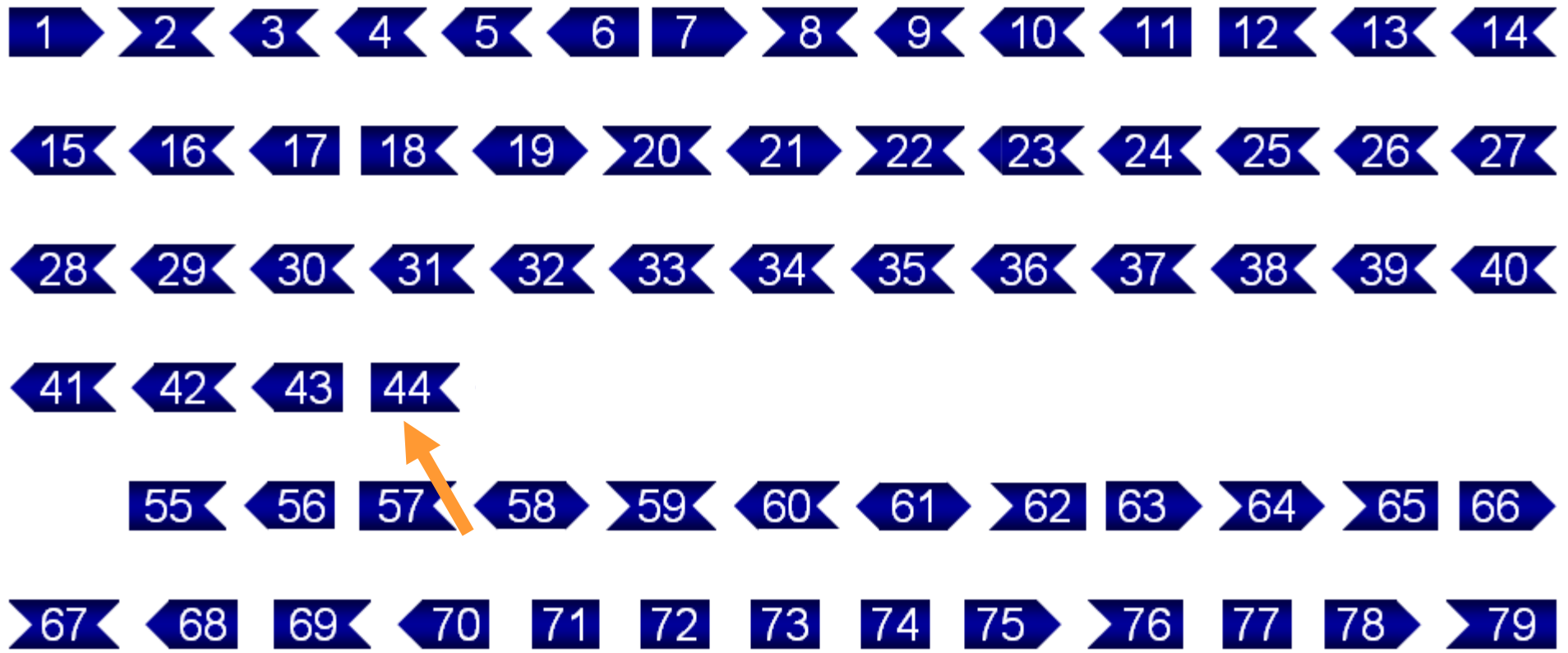


NT

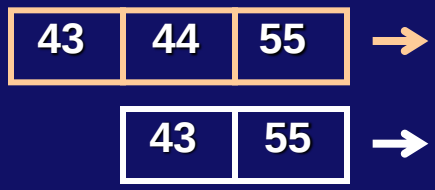
48 post transfection

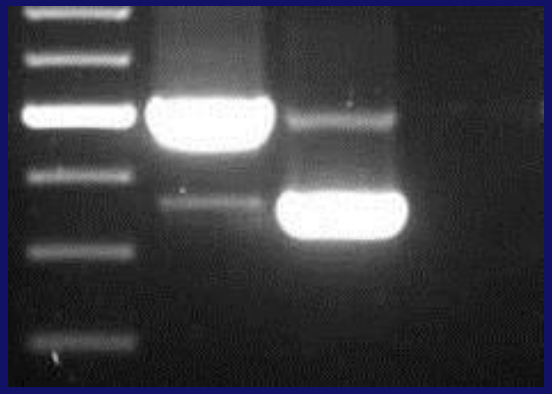


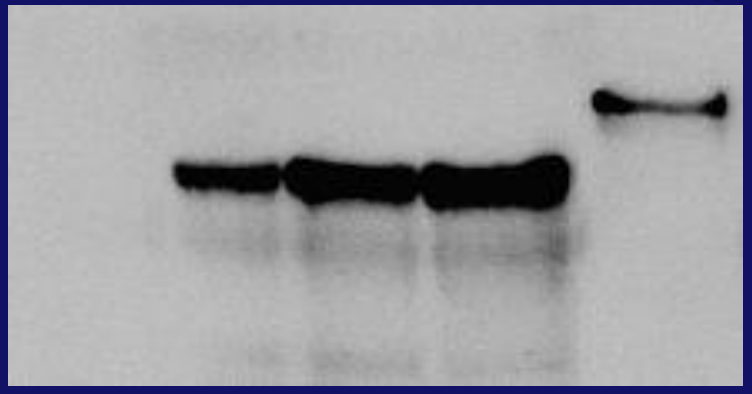
Exon 44 skipping in Δ exon 45-54 cells



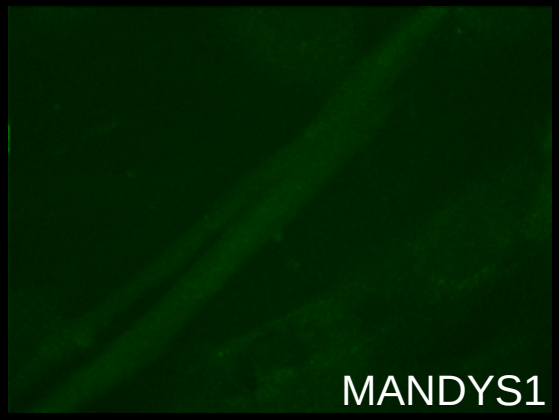
Exon 44 skipping in Δ exon 45-54 cells



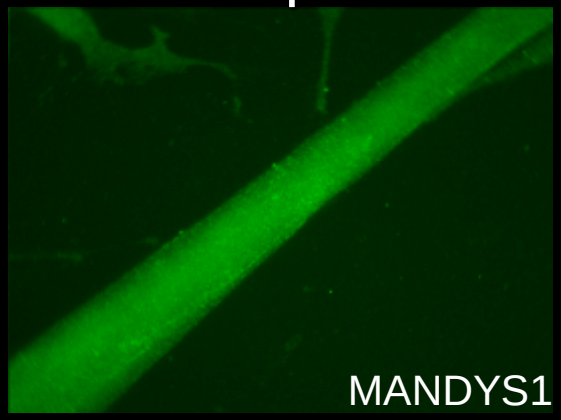


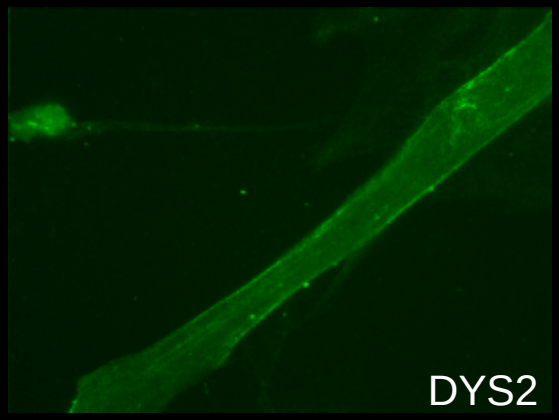


NT



48 hours post transfection

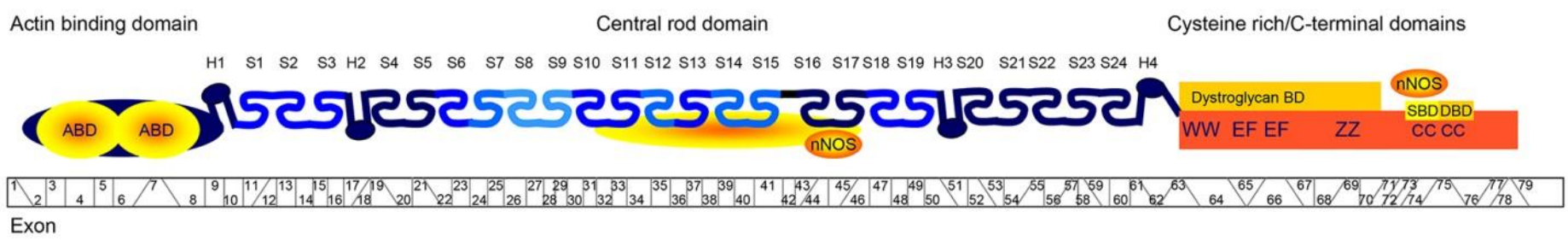




Therapeutic applicability confirmed for:

• Duplication exon 44	Skip **43 + 44**
• Deletion exon 45-54:	Skip **44**
• Duplication exon 45:	Skip **45**
• Deletion exon 45:	Skip **46**
• Point mutation exon 49:	Skip **49**
• Deletion exon 51-55:	Skip **50**
• Deletion exon 45-50, 48-50, 49-50, 50, 52:	Skip **51**
• Deletion exon 52:	Skip **53**
• Point mutation exon 43:	Skip **43** + **44**
• Deletion exon 46-50:	Skip **45** + **51**

Applicability



hotspot

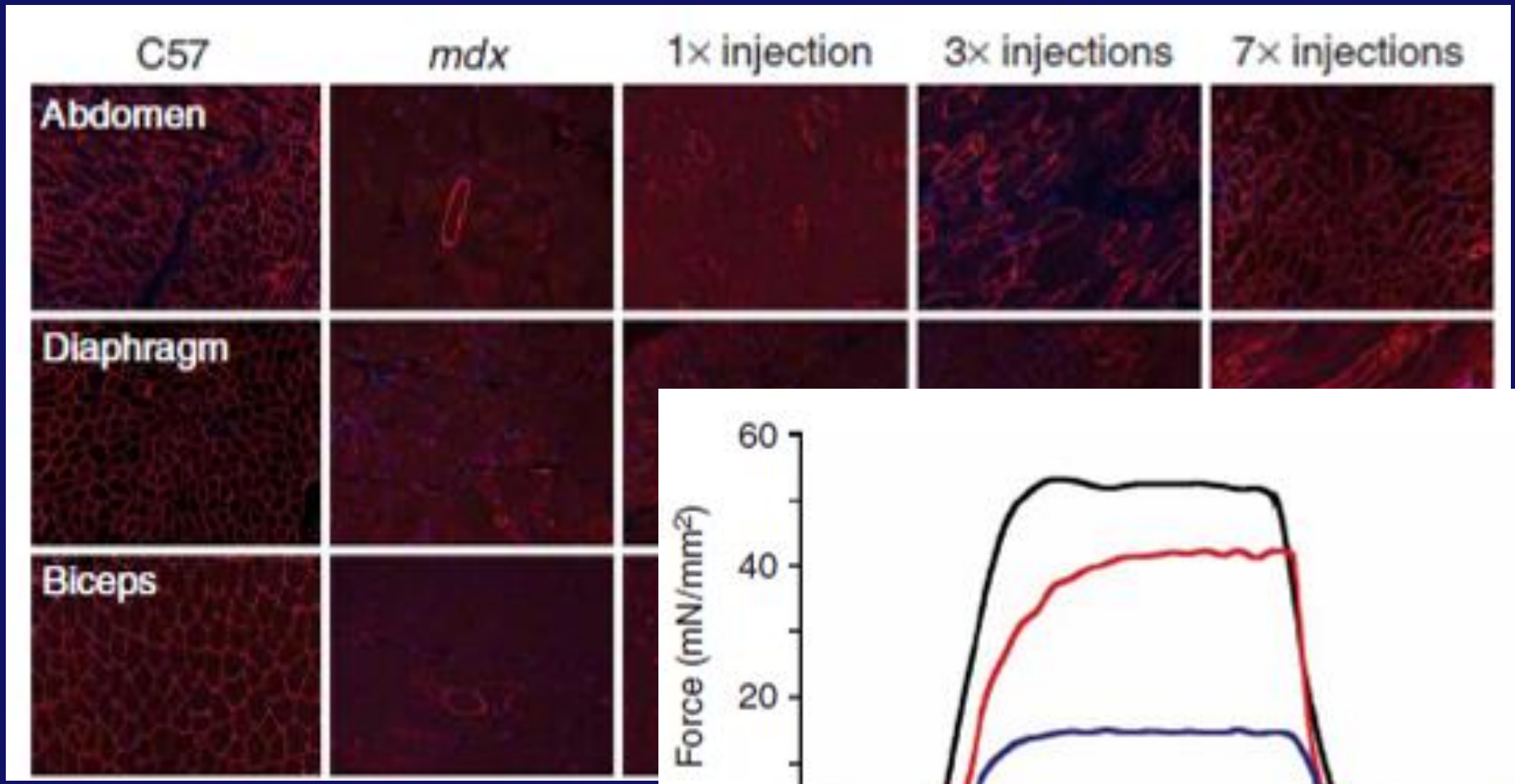
Exon	All mutations	Deletions
51	14%	21%
45	9.0%	13%
53	8.1%	12%
44	7.6%	11%
50	3.8%	5.6%
43	3.1%	4.5%
8	2.0%	2.9%

Bladen et al, Hum Mut 2015

The *mdx* mouse

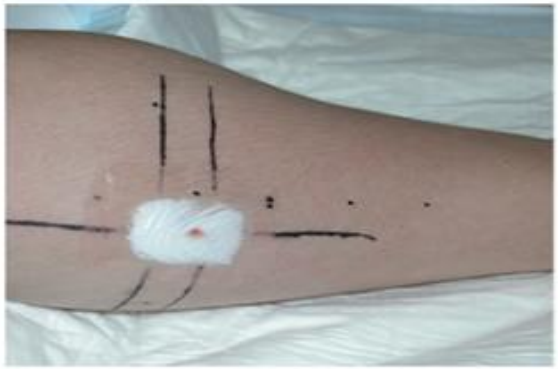
- Mutation in exon 23 ➔ No dystrophin
- Muscle is dystrophic, CK elevated
- Muscle function impaired
- Needs exon 23 skipping

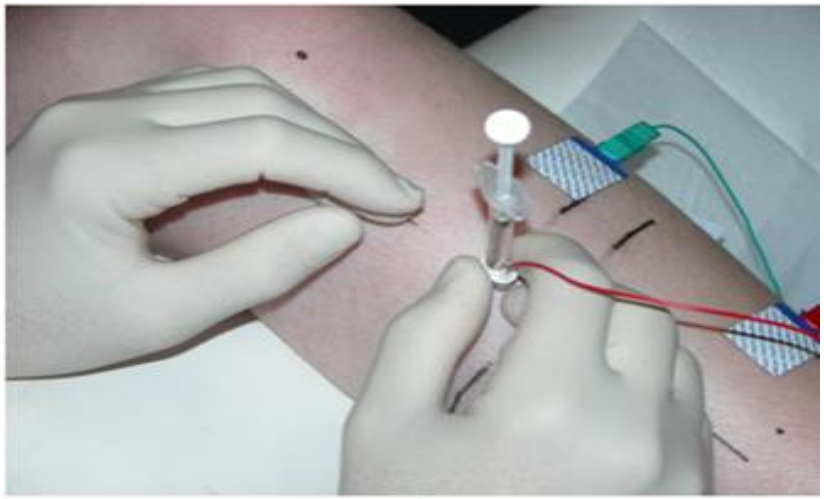
Exon skipping *mdx* mouse

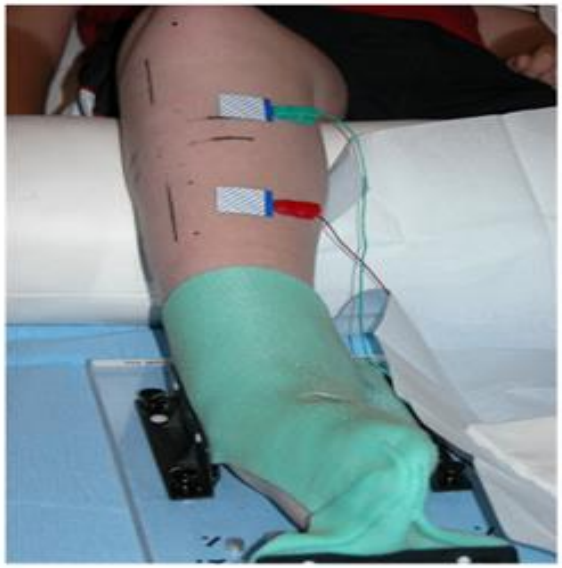


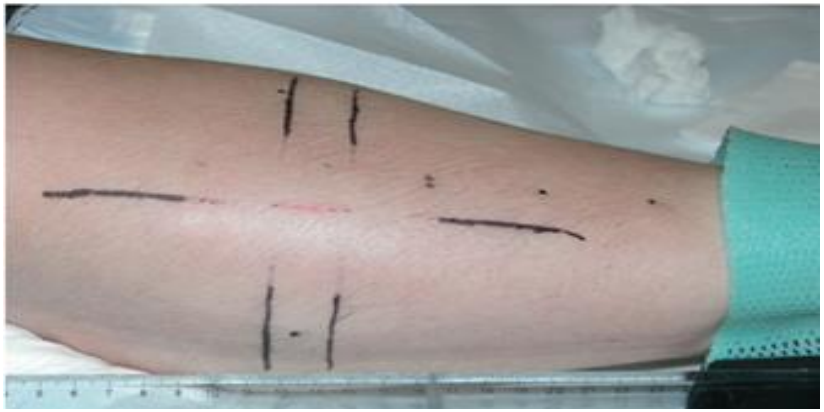
Clinical Development

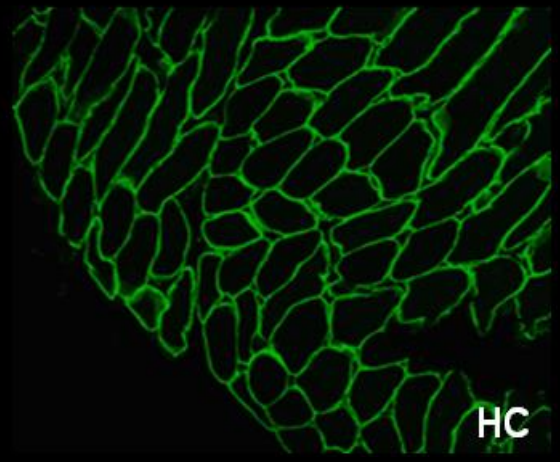
AON injection

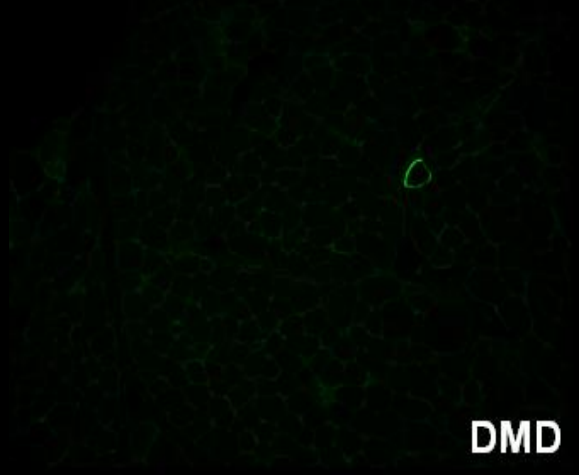






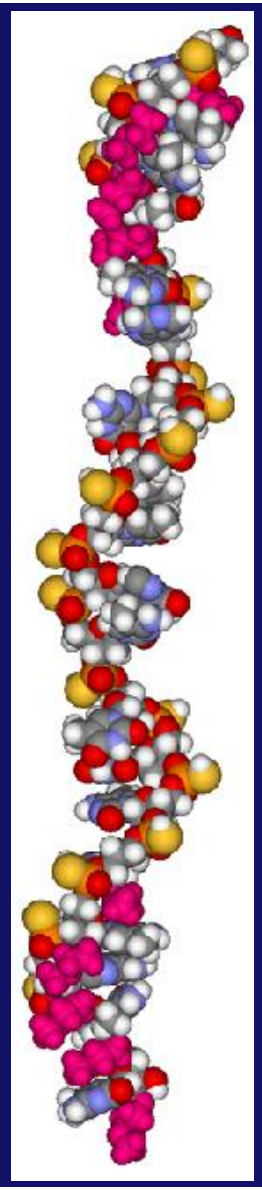



HC


DMD

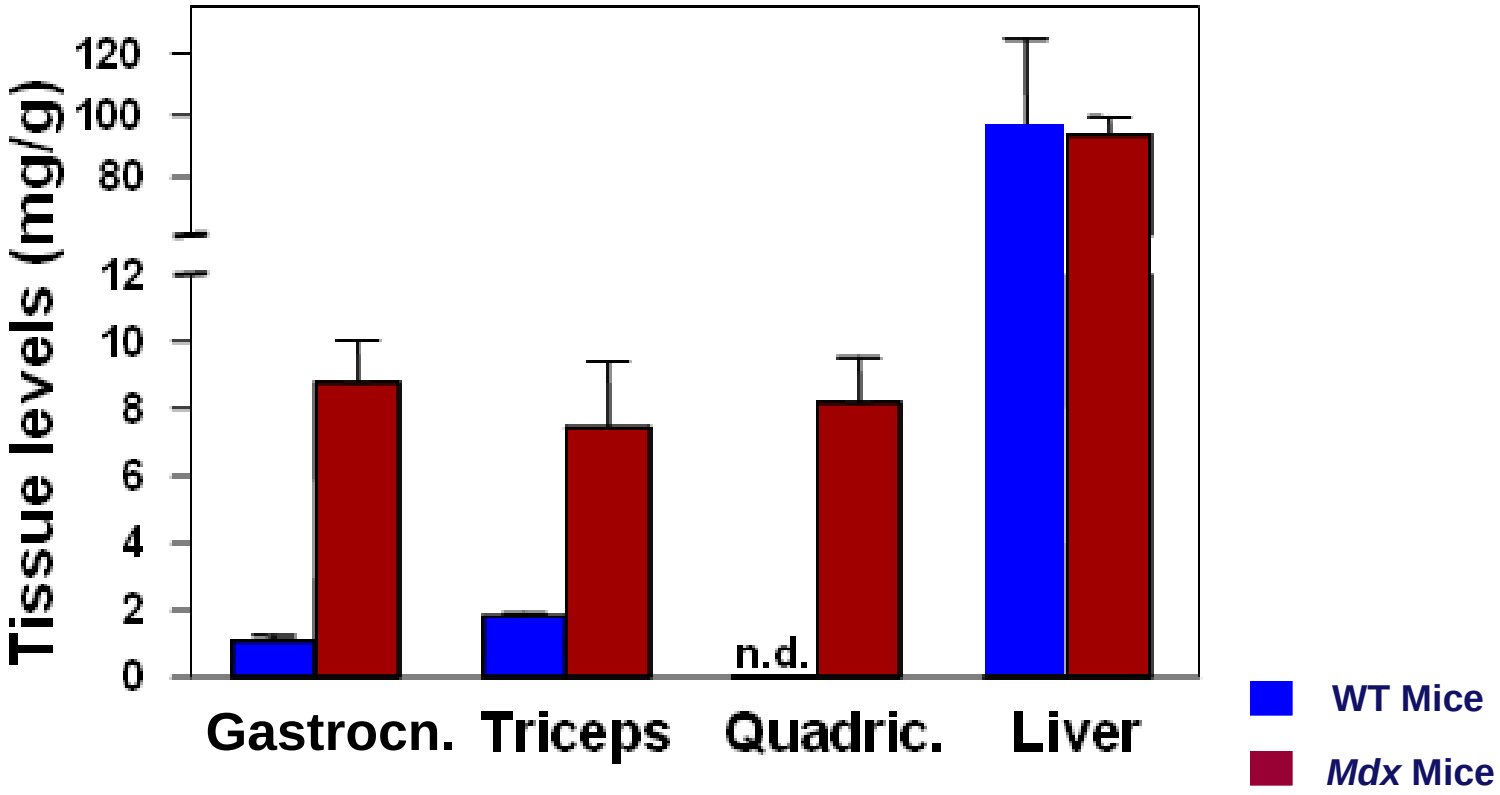
Considerations for AON delivery

- AONs very small (~8-12 kDa)
- Filtered out by kidney
- Phosphorothiate modification
  - Serum protein binding
  - Less clearance by kidney
  - Uptake by liver
- Uptake muscle poor, heart very poor



Systemic studies in *mdx* mouse model

AON levels in muscle and Liver



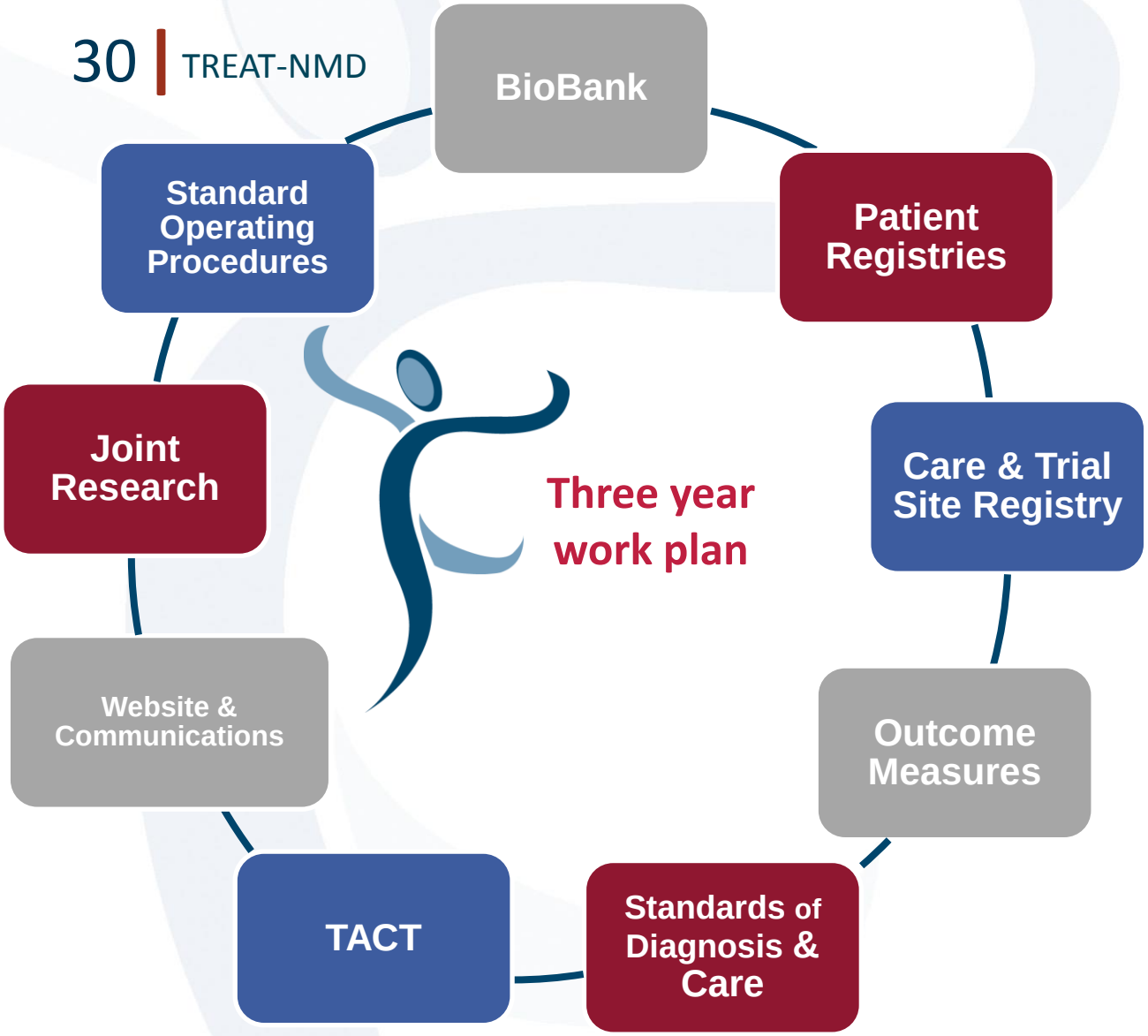
How are drugs approved?

- Regulators approve drugs based on a positive benefit/risk analysis
- Pharmacodynamic marker: dystrophin restoration
- Need to show ‘Clinical benefit’
- Outcome measure correlating with clinical benefit
- Cohorts where clinical benefit can be identified
- Natural history selected OM (power calculations)

Infrastructure needed for clinical trials

- Natural history data
- Outcome measures
- Biomarkers
- Rare disease ➔ multiple trials sites needed
  - Care standards
  - Expert centers for DMD
- Personalized approach
  - Registries to identify eligible patients

TREAT-NMD
Neuromuscular Network



TREAT-NMD
Neuromuscular Network

2007-2011
EU funded Network

2012 onwards
Alliance funded through multiple streams with global partners & membership

Governance
Chair – Annemieke Aartsma-Rus
Vice Chair – Eric Hoffman

Executive Committee
Supported by academic advisory board ("task force") of NMD leaders

www.treat-nmd.eu

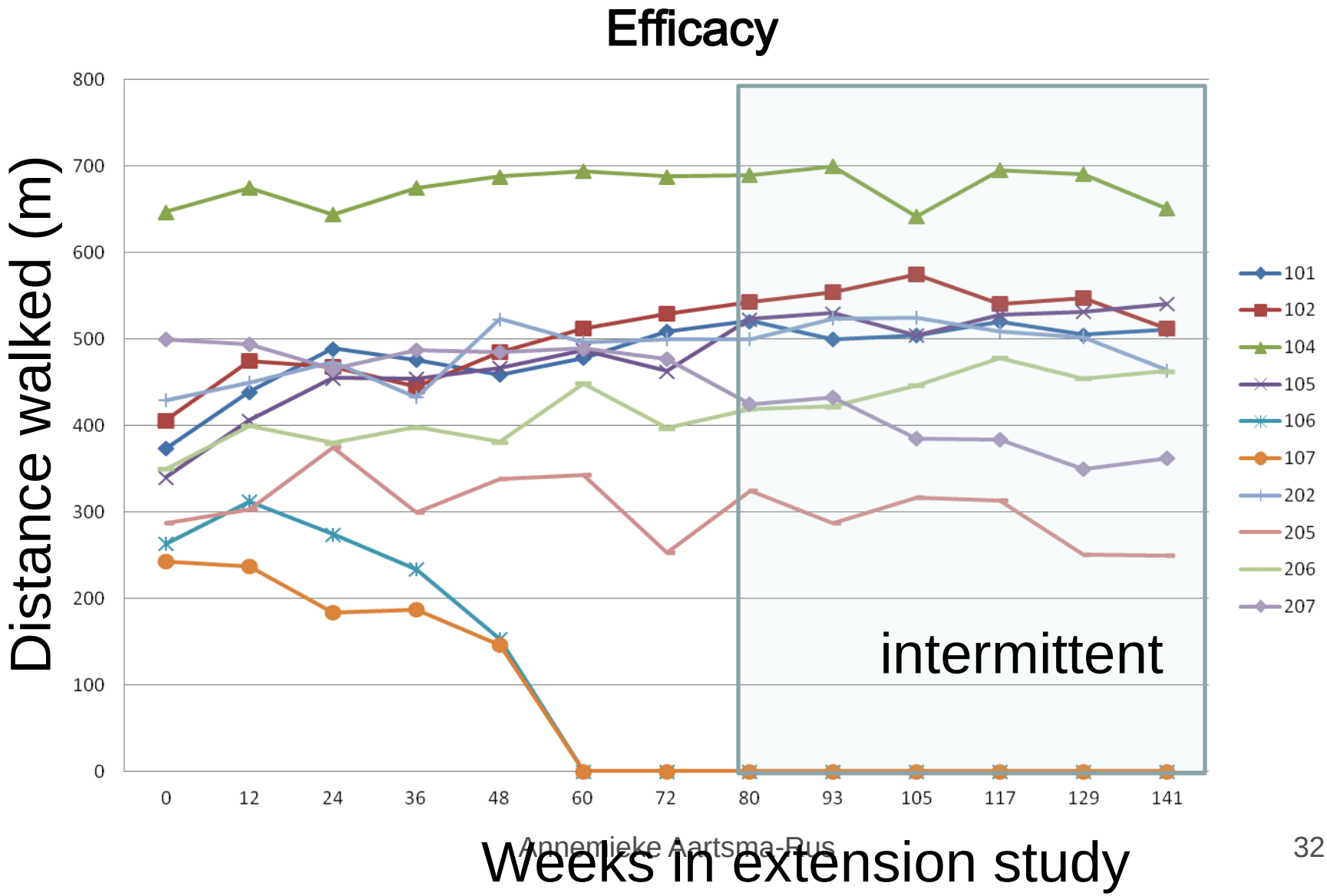
Meanwhile: trials were initiated

- Dose escalating study (0.5 – 6 mg/kg/week for 4 weeks
- Dystrophin restored in 10/12 patients
- All patients then enrolled in an open label extension study
- 6 mg/kg drisapirsen per week for 72 weeks
- Then 8 weeks break
- Then cycles of 8 weekly doses, 4 week break
- Treated for almost 4 years

PROSENSA

Goemans et al, NEJM 2011, 364: 1513-22

PRO051-02 / DMD114673



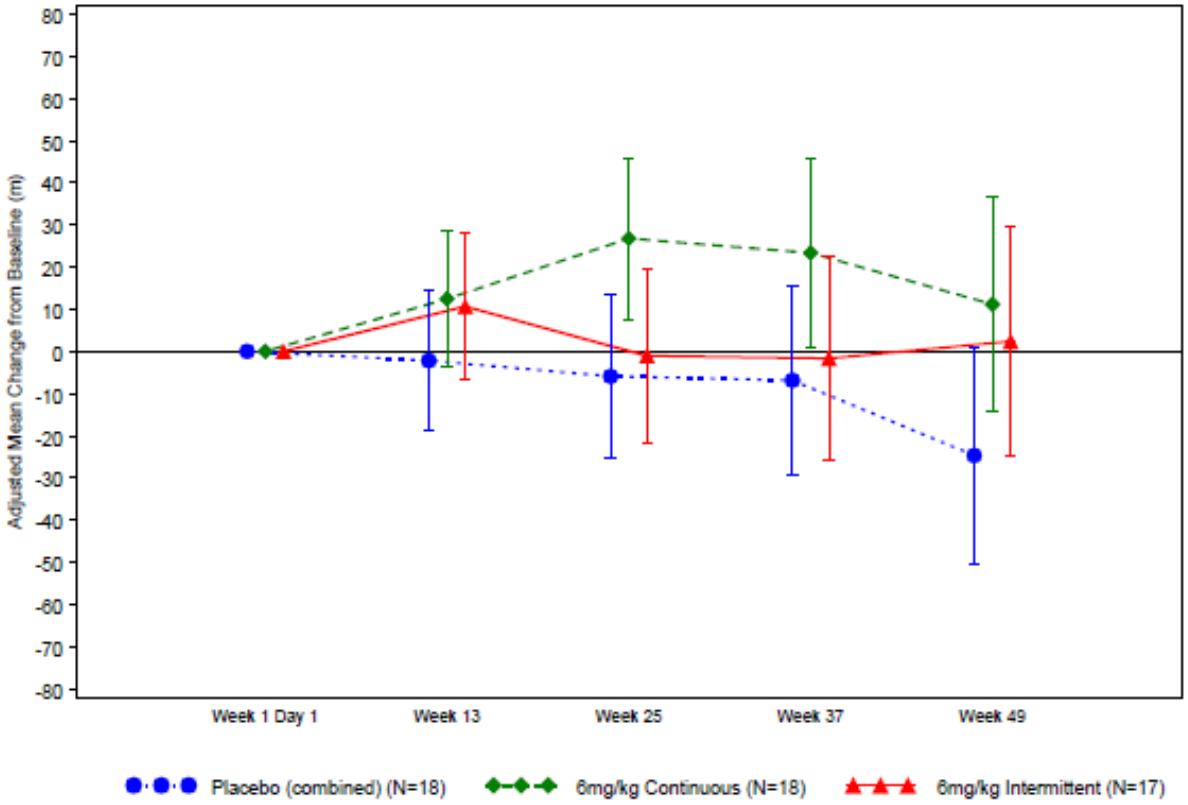
Open Label Extenion Trial Drisapersen

- Side effects observed
  - Local injection site reactions
  - Proteinuria (reversible during breaks)
  - Thrombocytopenia seen for some patients
  - Seen in all phase 2/3 trials drisapersen
- 6 minute walk distance maintained for >3.5 years in 8/10 ambulant patients
- Very encouraging results but no placebo group

Placebo-controlled trial drisapersen

- 54 patients
- Early stage of disease
- Steroid treated
- Treatment for 48 weeks with 6 mg/kg drisapersen subcutaneous or placebo
  - Weekly injections (18 patients)
  - Intermittent regimen (17 patients)
  - Placebo (18 patients)

Primary Endpoint: Change from Baseline (95% CI) in 6MWD (m), ITT Population



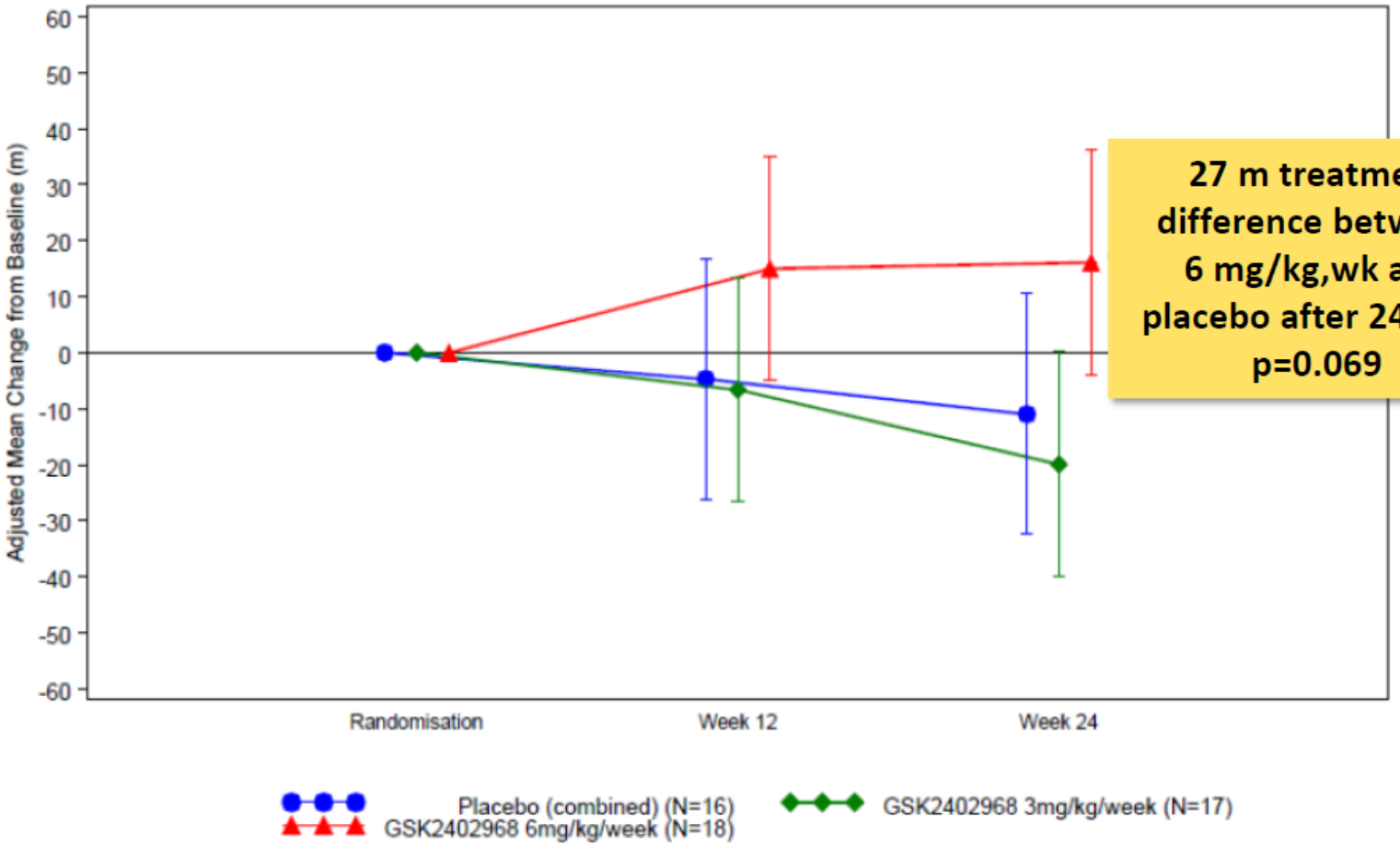
Visit	Comparison	Treatment Difference	P-value
Week 25 (Primary)	Weekly vs Placebo	35.09	0.014
	Intermittent vs Placebo	3.51	0.801
Week 49 (Secondary)	Weekly vs Placebo	35.84	0.051
	Intermittent vs Placebo	27.08	0.147

Placebo-controlled trial drisapersen

- 51 patients
- Early stage of disease (rise from floor <15 seconds)
- Steroid treated
- Treatment for 24 weeks with 3 or 6 mg/kg drisapersen subcutaneous or placebo
- 24 week wash out

Drisapersen DEMAND V (DMD114876)

Adjusted Mean Change from Baseline (95% CI) in 6MWD (m) – Primary MMRM analysis
ITT Population



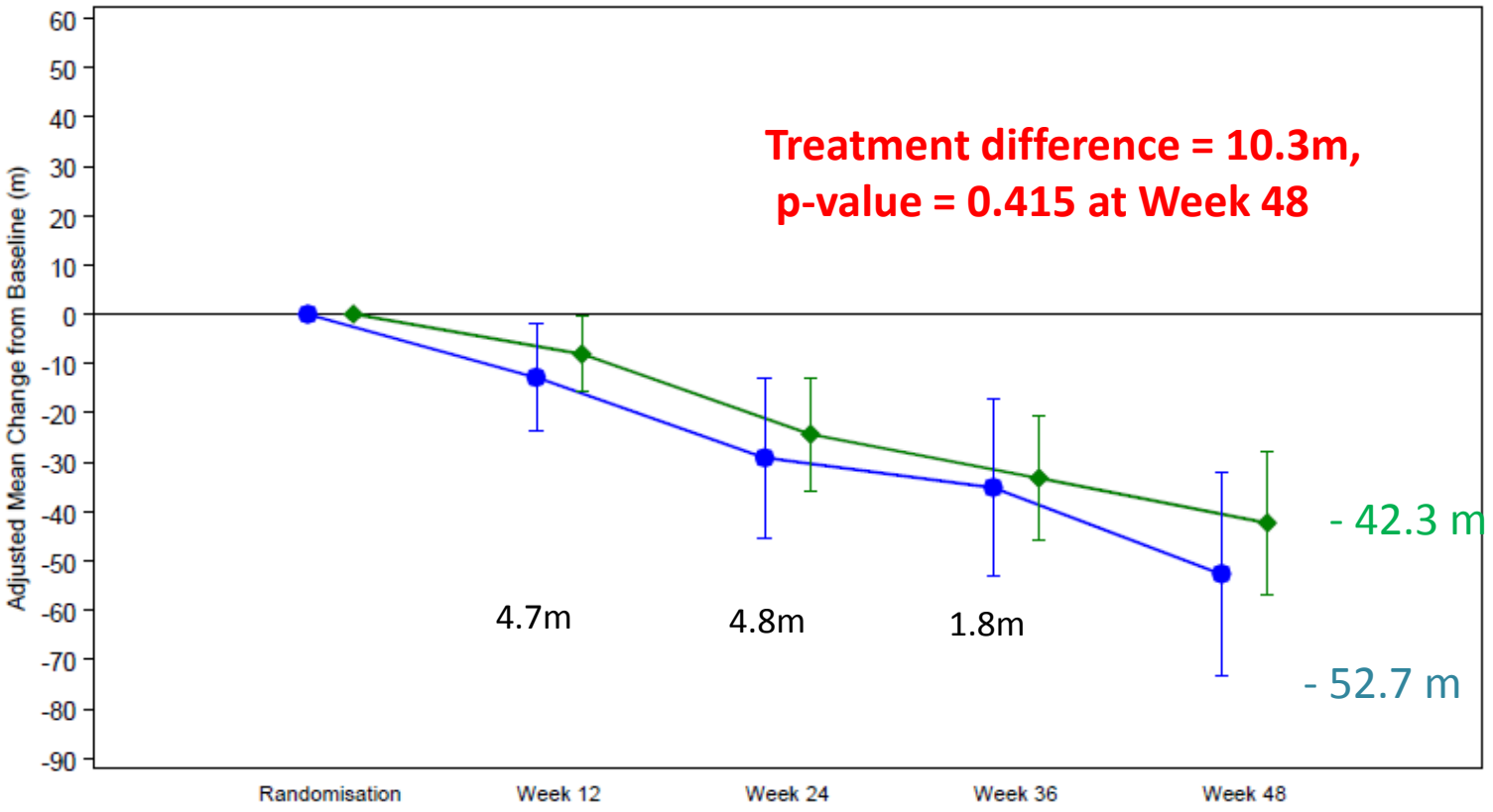
27 m treatment difference between 6 mg/kg,wk and placebo after 24 wks; p=0.069

Placebo study drisapersen

- Phase 3 (48 weeks)
  - Patients 5-16 years old (186)
  - Global study
  - 6 mg/kg/week or placebo

Primary Endpoint: 6MWD

Adjusted Mean Change from Baseline (95% CI) in 6MWD (m) – Primary MMRM analysis
ITT Population



Curves offset for visualization

Placebo (N=61) GSK2402968 6mg/kg/week (N=125)

n	61	58	59	60	59
	125	124	122	116	117

Model includes terms for Treatment, Visit, Treatment by Visit, Country Grouping, Baseline 6MWD and Baseline 6MWD by Visit.
A postitive change from baseline indicates improvement.

Bad News

PRESS RELEASE

Issued: Friday 20 September 2013, London UK, Leiden, Netherlands

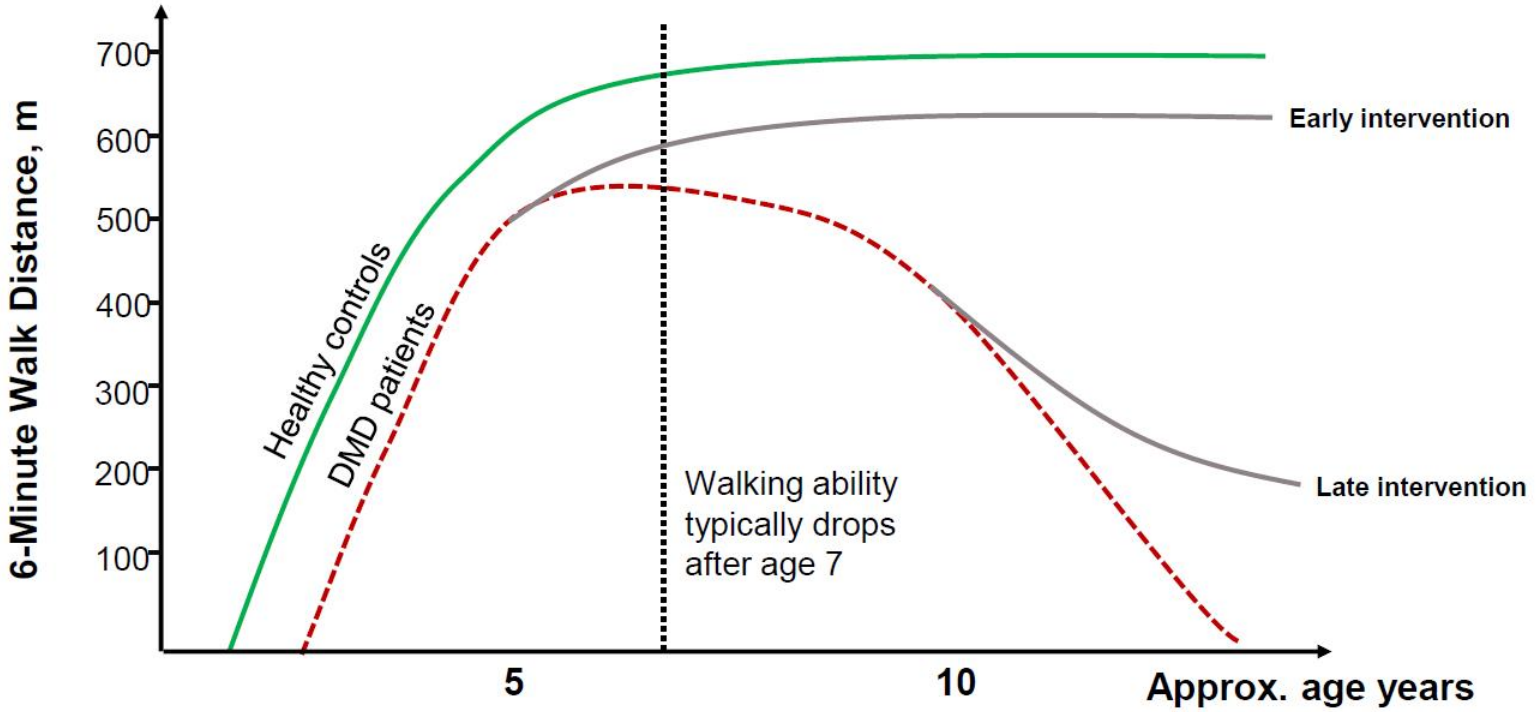
GSK and Prosensa announce primary endpoint not met in Phase III study of drisapersen in patients with Duchenne Muscular Dystrophy

GlaxoSmithKline (GSK) and Prosensa today announced that GSK's Phase III clinical study of drisapersen, an investigational antisense oligonucleotide, for the treatment of Duchenne Muscular Dystrophy (DMD) patients with an amenable mutation, did not meet the primary endpoint of a statistically significant improvement in the 6 Minute Walking Distance (6MWD) test compared to placebo.

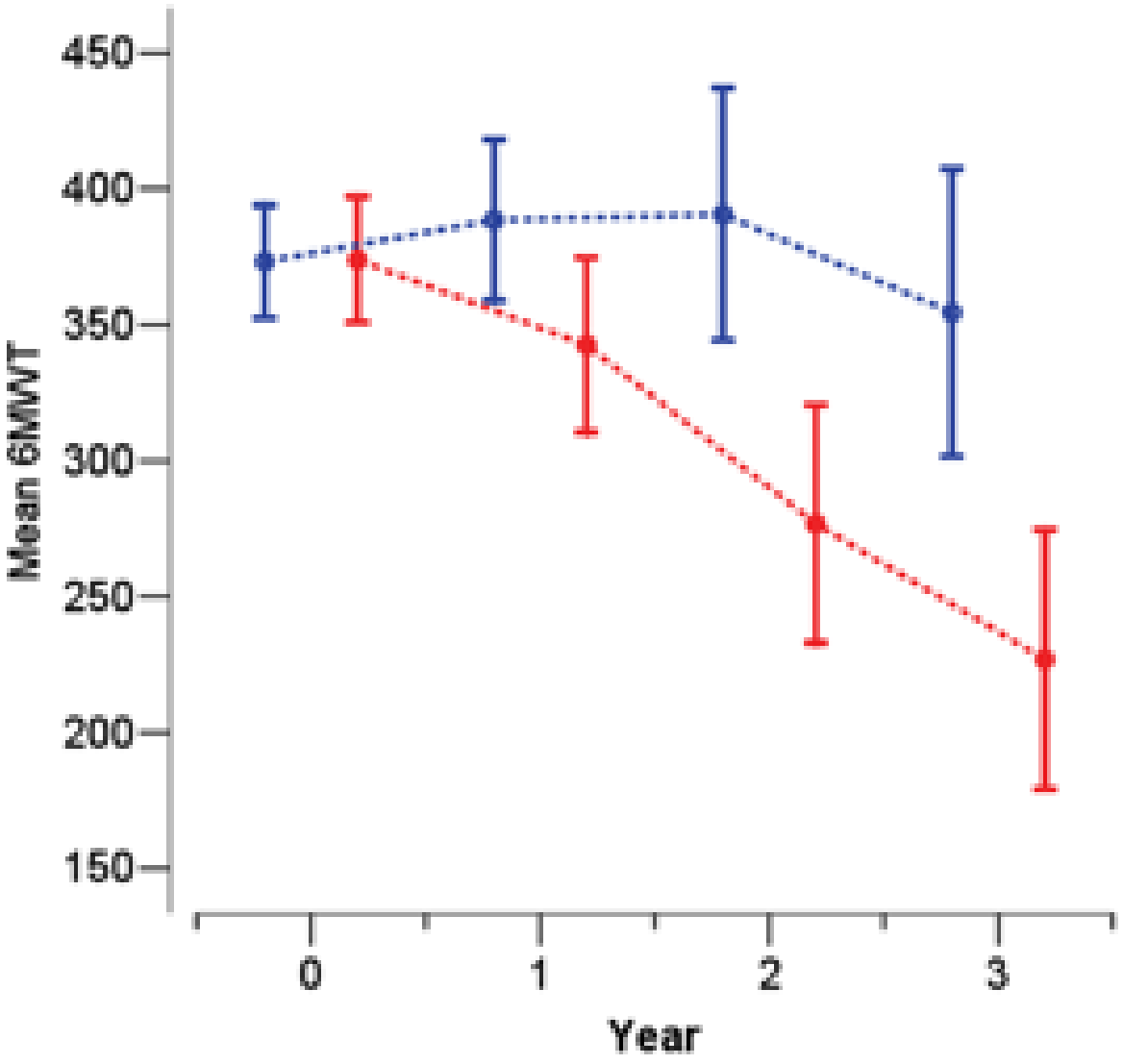
What we know now

Functional Outcome - 6 Minute Walk Test

Conceptual representation of 6MWT performance by DMD patients and healthy controls



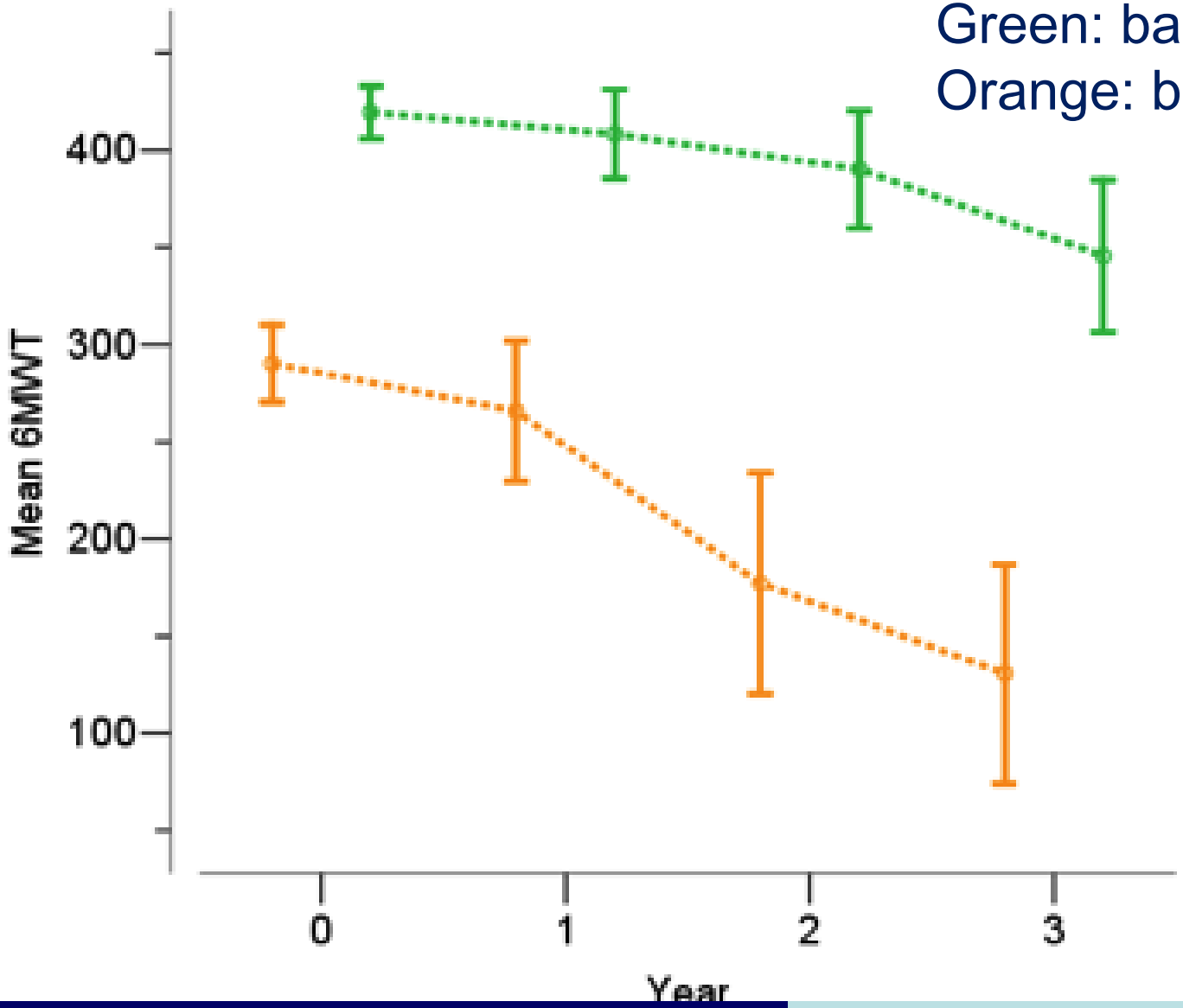
Influence of age



Blue: below 7
Red: above 7

Influence of disease stage

Green: baseline >350 m
Orange: baseline <350 m



Drisapersen preliminary further analysis

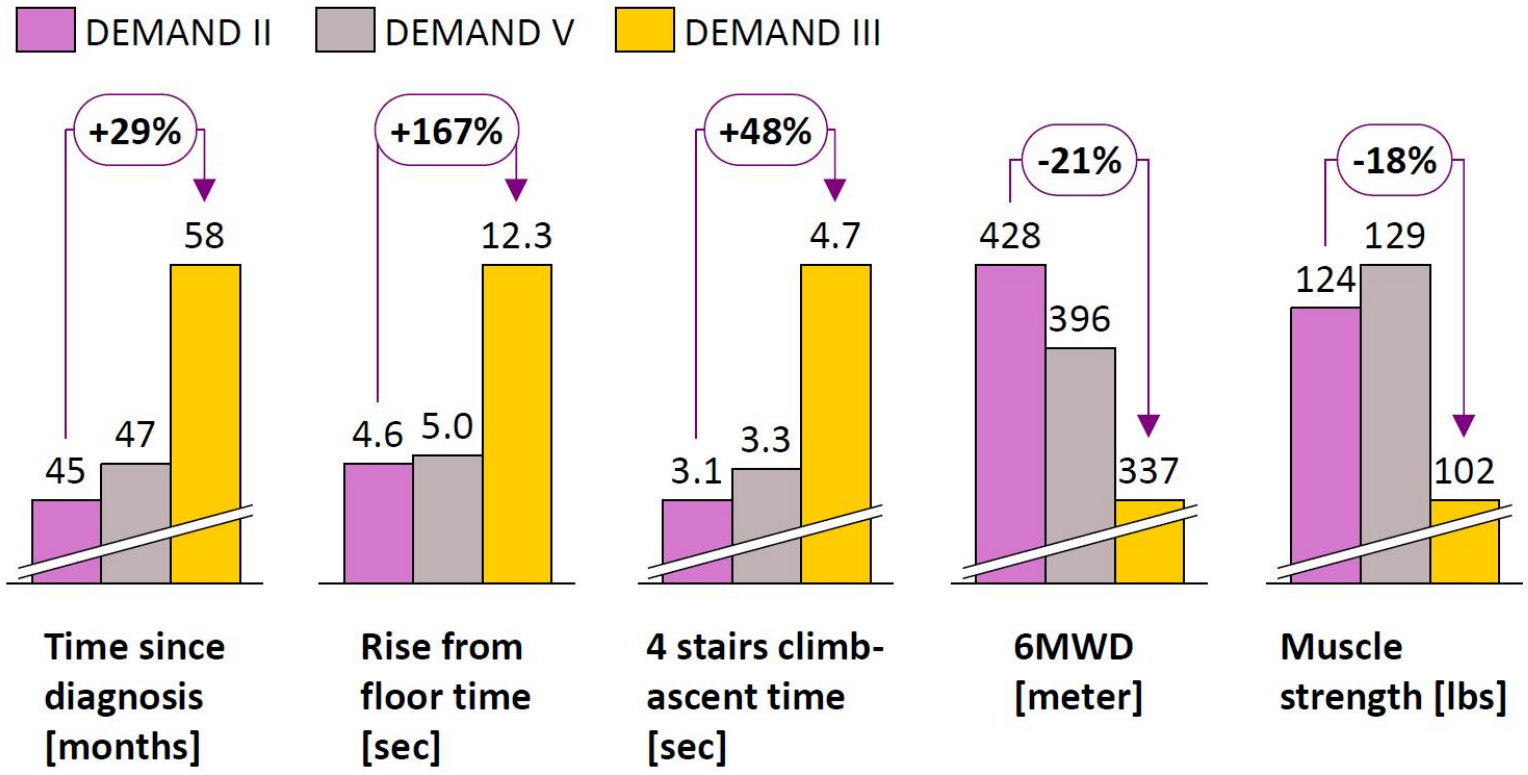
DEMAND III included older patients…

Study	Treatment	Age (yrs), mean (sd), range
DEMAND II DMD114117	Placebo	6.9 (1.2) 5-9
	Drisapersen	7.2 (1.7) 5-11
DEMAND V DMD114876	Placebo	8.0 (1.8) 5-11
	Drisapersen	7.6 (2.7) 5-13
DEMAND III DMD114044	Placebo	8.0 (2.4) 5-16
	Drisapersen	8.3 (2.4) 5-16

Drisapersen preliminary further analysis

...with more advanced disease...

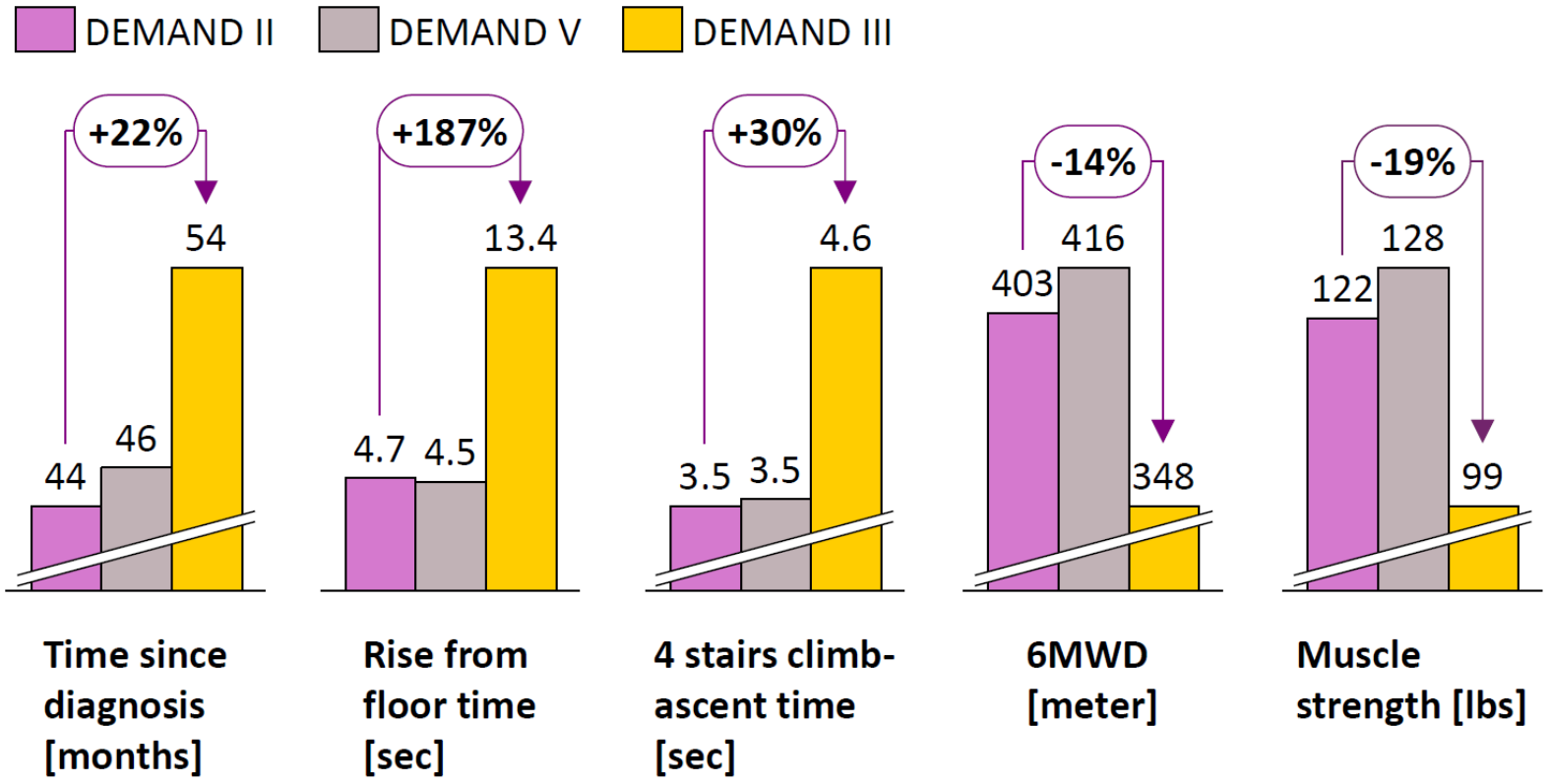
The drisapersen arm boys in DEMAND III were generally more advanced in their disease



Drisapersen preliminary further analysis

...in both treatment arms

The placebo arm boys in DEMAND III were generally more advanced in their disease



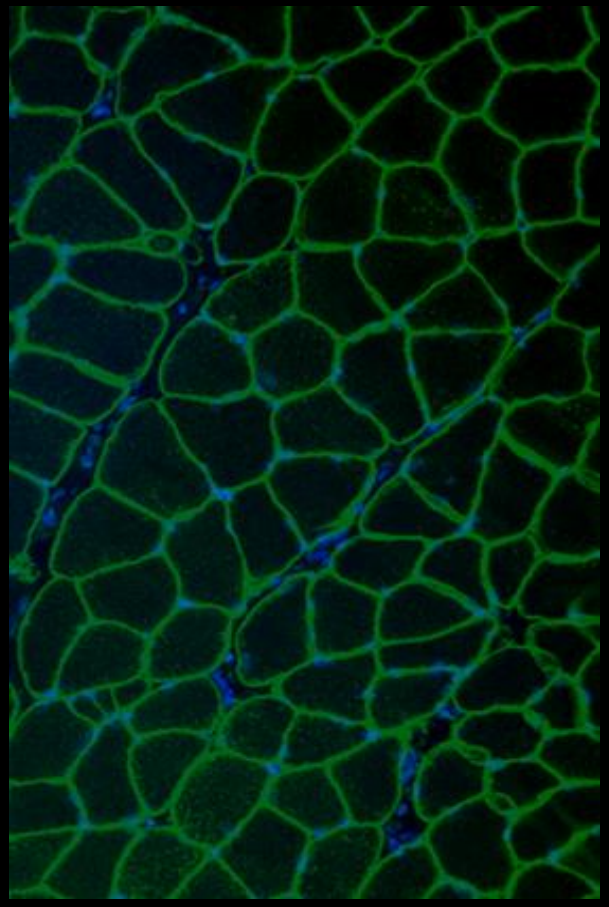
In hindsight

- Information too limited to allow set up ideal trial
- Limited information on 6MWT
  - Variation
  - Progression in different age ranges
- Power calculations impossible
- Selection of ideal cohort impossible

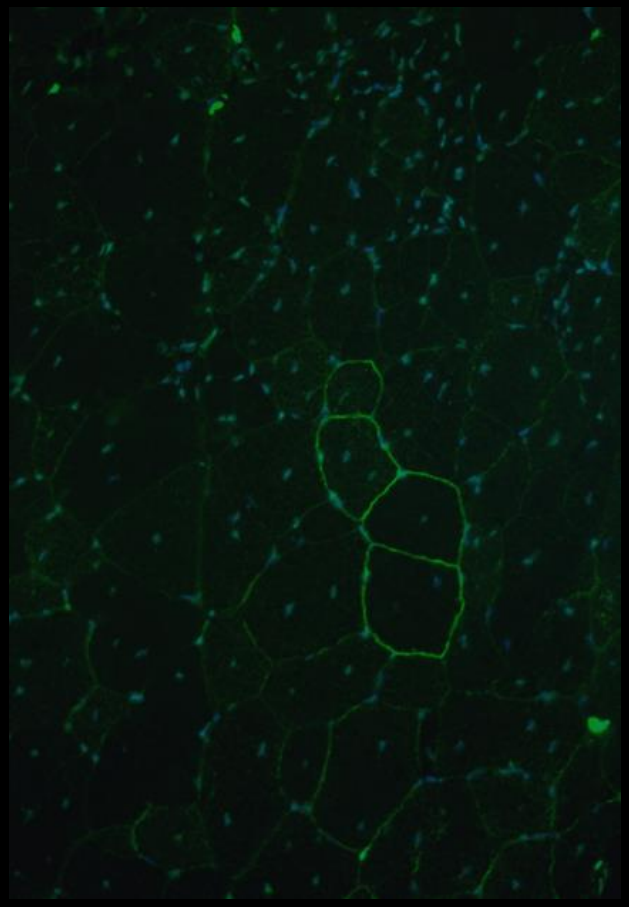
Biochemical OM: dystrophin

Dystrophin immune staining

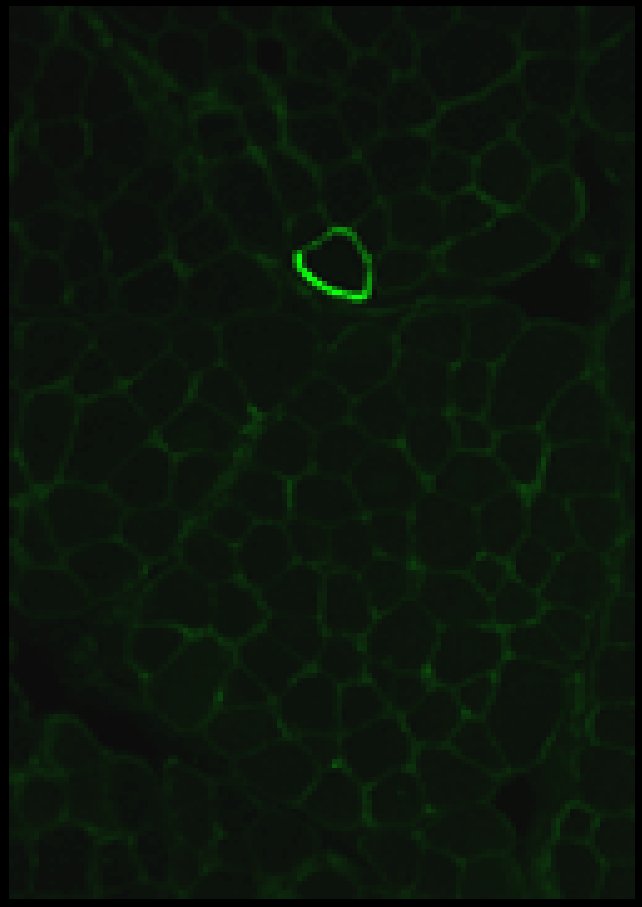
Healthy



Mdx mouse



Duchenne



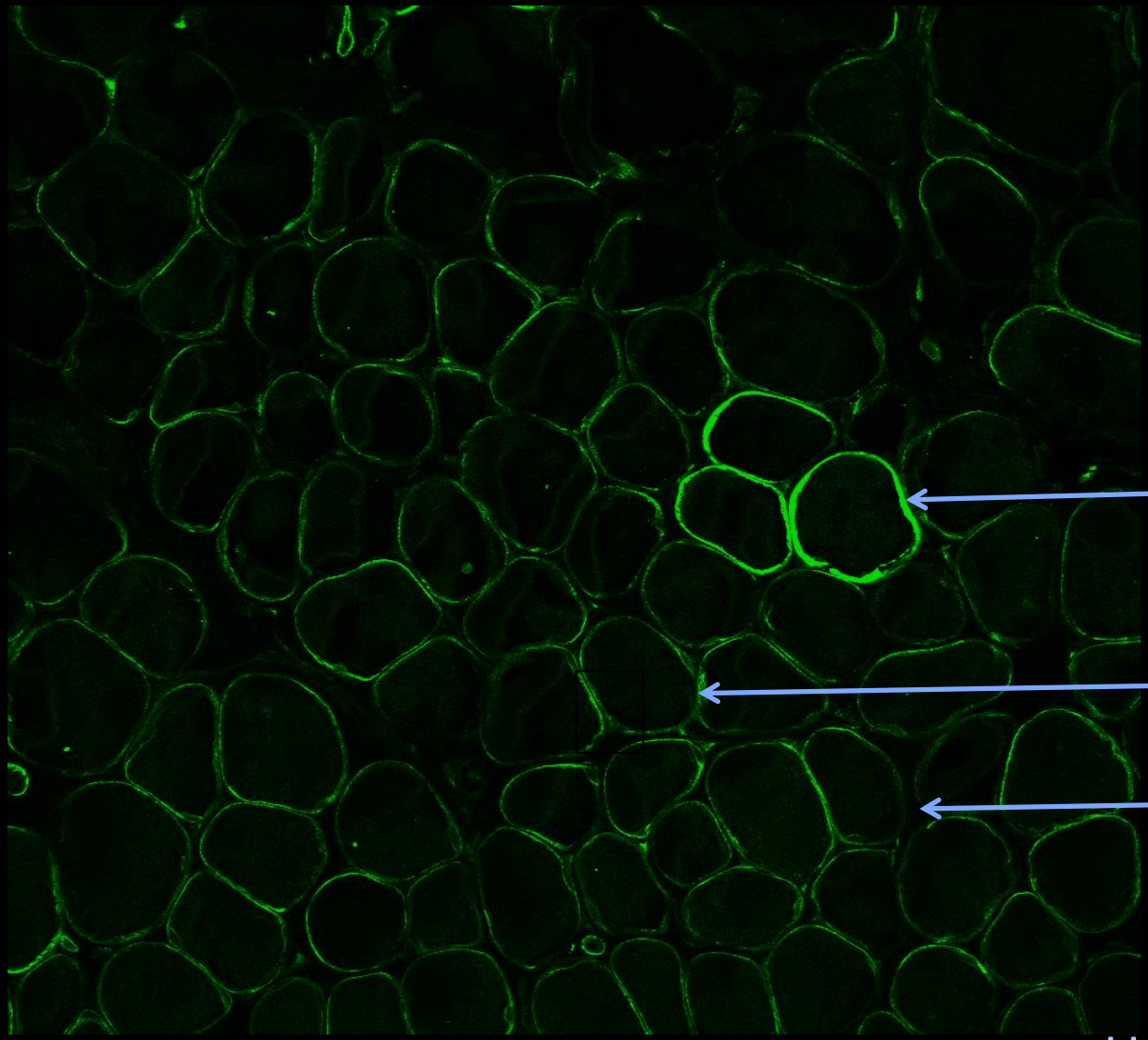
Dystrophin restoration

- Showing dystrophin restoration is not enough for drug approval
- Clinical benefit measured by functional outcome
- Functional effect of dystrophin restoration will depend on time of intervention
- Pharmacodynamic biomarker
  - Did the treatment work as proposed?
  - Confirmation drug mechanism

Dystrophin analysis

- Historically
  - <3% dystrophin: DMD
  - >20% dystrophin: BMD
- Based on older, less sensitive and less reliable techniques to quantify dystrophin levels
- More sensitive and sophisticated techniques have been developed

Revertant fibers & trace amounts



Untreated DMD muscle

New insights

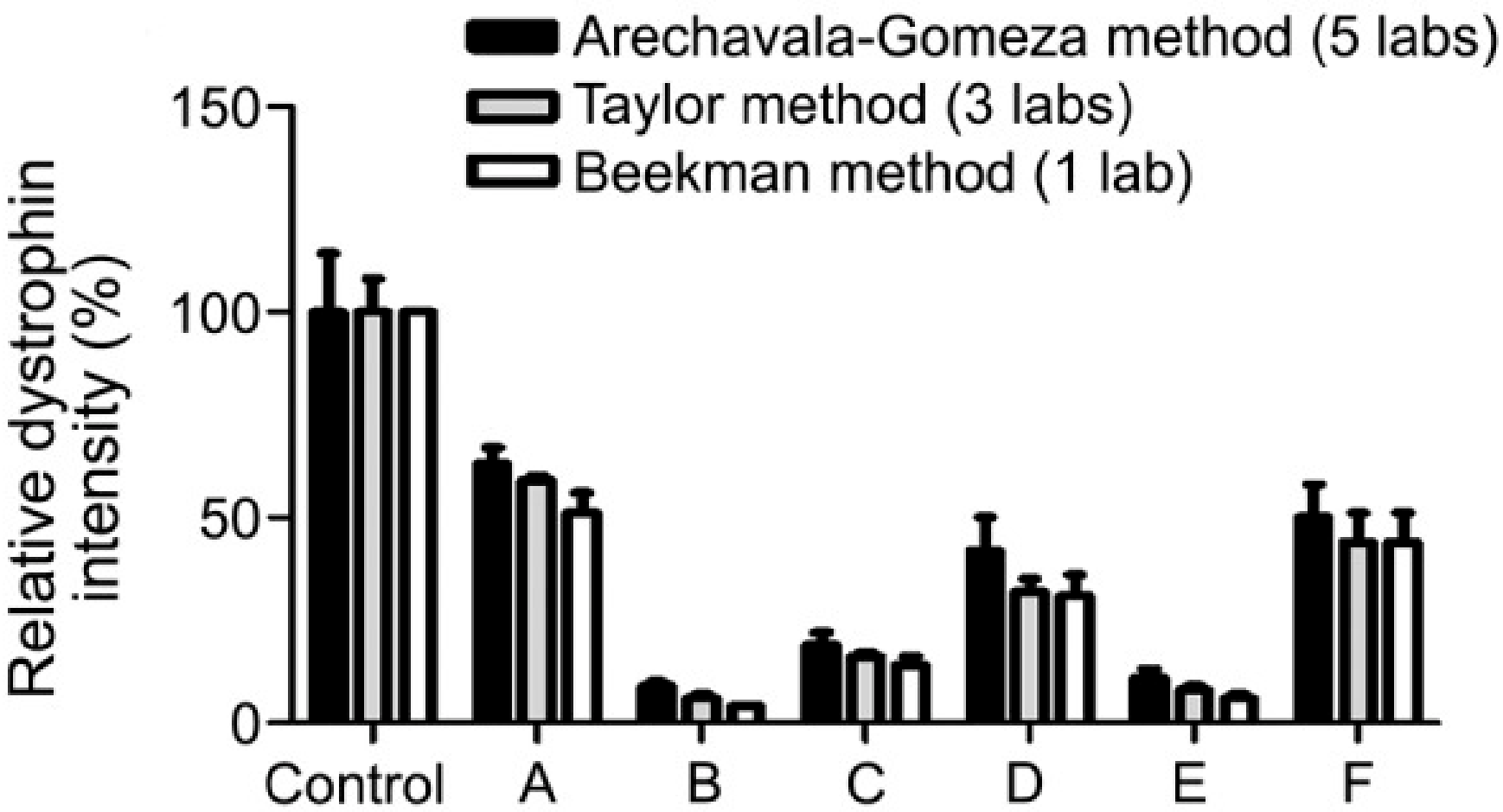
- <20% dystrophin can lead to BMD[1]
- Most DMD patients make SOME dystrophin
  - Revertant fibers (individual fibers, high levels)
  - Trace dystrophin (most fibers, very low levels)
- Very low dystrophin levels (exon 44 skippable DMD patients[2)]
  - Slower decline 6MWT[3]
  - Higher age at loss of ambulation[4]

1. JNNP 2014; 85:747-53; 2: JAMA Neurol 2014; 71: 32-40 3: PLoS ONE 2014; e83400; 3: JND 2014; 1: 91-95

Challenges dystrophin analysis

- Difficult to measure
- Need pre- and post treatment biopsies
- How reproducible is analysis
- How comparable between different labs?

Dystrophin analysis comparison



Anthony et al Neurology 2014

This is not the end

Drisapersen

- Phased redosing patients started Q3 2014
- Biomarin filed for accelerated approval with FDA (April 2015)
- Prepare for filing conditional approval with EMA

However, supoptimal trial caused delays!

Take home messages

- After showing proof-of-concept for therapeutic approach, set up tools for biochemical outcome measures and functional outcome measures and collect natural history data in parallel with preclinical studies
- Involve patients in development functional outcome measures from the start
- Involve regulators in development outcome measures from an early stage

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