Leids Universitair Medisch Centrum

Exon skipping therapy for Duchenne muscular dystrophy
It takes more than an antisense oligonucleotide

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Disclosures

- Employed by LUMC, which has patents on exon skipping technology, some of which has been licensed to BioMarin and subsequently sublicensed to Sarepta. As co-inventor of some of these patents I am entitled to a share of royalties
- Ad hoc consultant for PTC Therapeutics, BioMarin Pharmaceuticals Inc., Alpha Anomeric, Eisai, Sarepta Therapeutics, Takeda, Regenxbio, Splicesense, Galapagos, Global Guidepoint and GLG consultancy, Grunenthal, Wave and BioClinica. Remuneration paid to LUMC
- Member of the scientific advisory boards of ProQR, Sarepta Therapeutics, Hybridize Therapeutics and Silence Therapeutics. Remuneration paid to LUMC
- LUMC received speaker honoraria from PTC Therapeutics and BioMarin Pharmaceuticals

European Reference Network for rare or low prevalence complex diseases

Network Neurological Diseases (ERN-RND)

ean european academy of neurology

European Reference Network for rare or low prevalence complex diseases

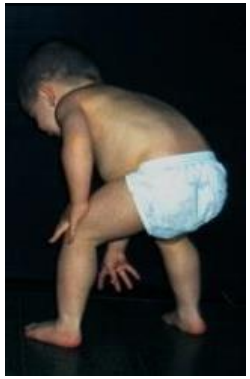
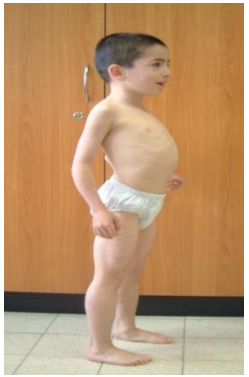
Network Neuromuscular Diseases (ERN EURO-NMD)

Learning objectives

By the end of this webinar you will be able to:

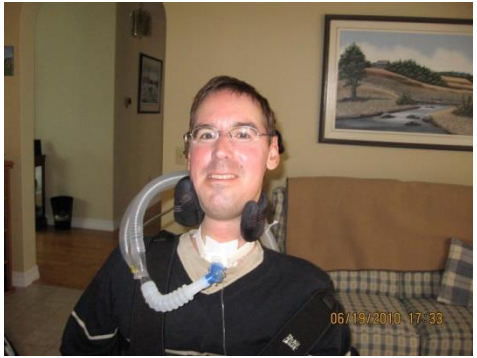
- Know the different steps of preclinical and clinical development of a therapy for a rare diseases
- Be aware of the challenges of rare disease therapy development
- Know the importance of involving all stakeholders in rare disease therapy development

Duchenne Muscular Dystrophy







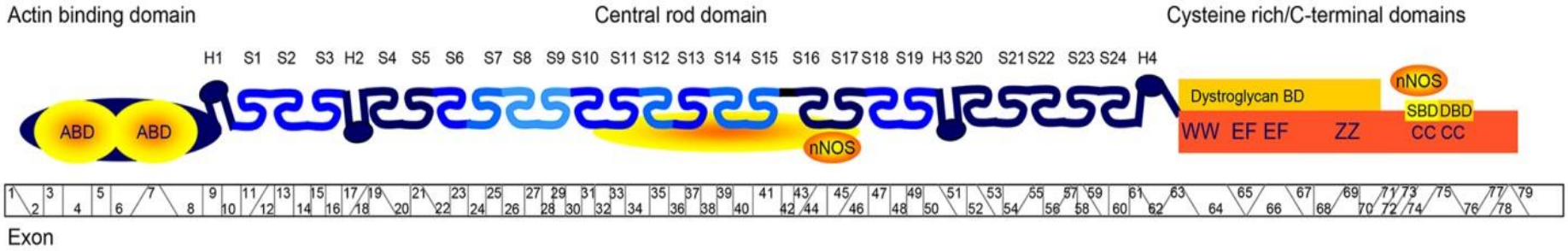
Question 1:

Q1. How do you think Duchenne patients rate their quality of life?

- Severely reduced compared to unaffected individuals
- Mildly reduced compared to unaffected individuals
- Comparable to unaffected individuals
- Better than unaffected individuals

Dystrophin protein

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



- Functional domains located at N- and C-terminal part of protein

Exon 48-50 deletion

Disrupted reading frame

↓

Protein translation truncated prematurely

↓

Dystrophin not functional

Becker: reading frame maintained

Reading frame not disrupted

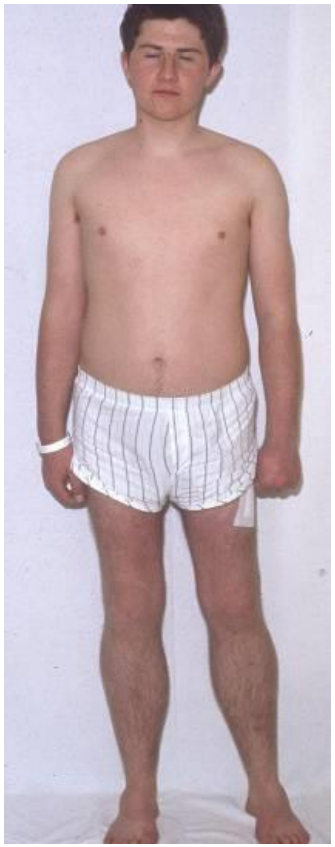
↓

Protein translation continues

↓

Dystrophin partly functional

Duchenne vs Becker



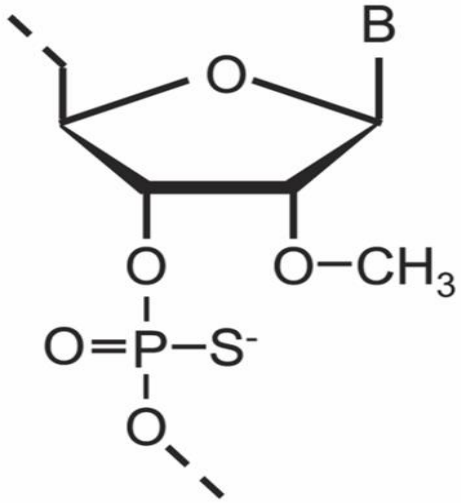
Exon skipping to restore reading frame

Reading frame restored

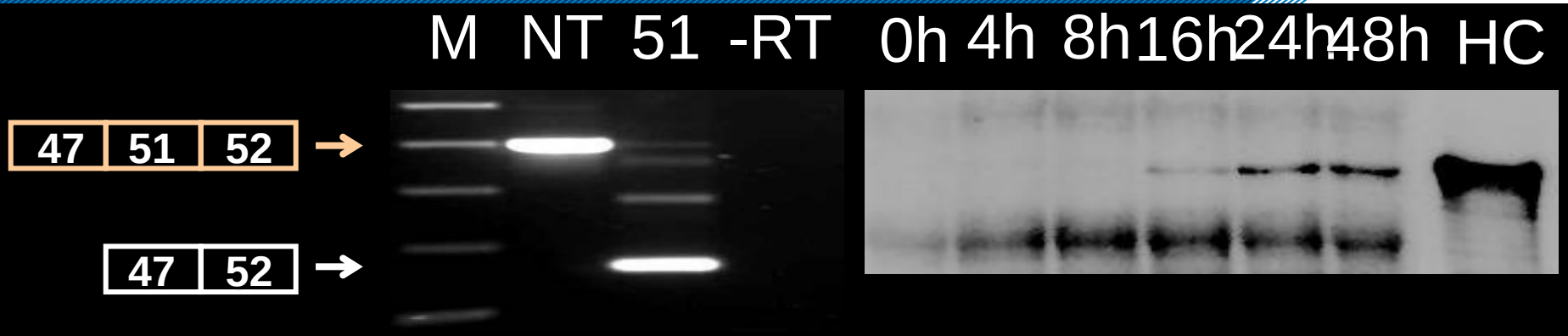
Partially functional dystrophin

Antisense oligonucleotides

- Requirements for splice modulating AONs
  - Nuclease resistant
  - Not induce RNase H
  - Good PK properties
- Many chemistries available
- Used 2’-O-methyl phosphorothioate
  - Stable
  - Cheap
  - Good PK properties

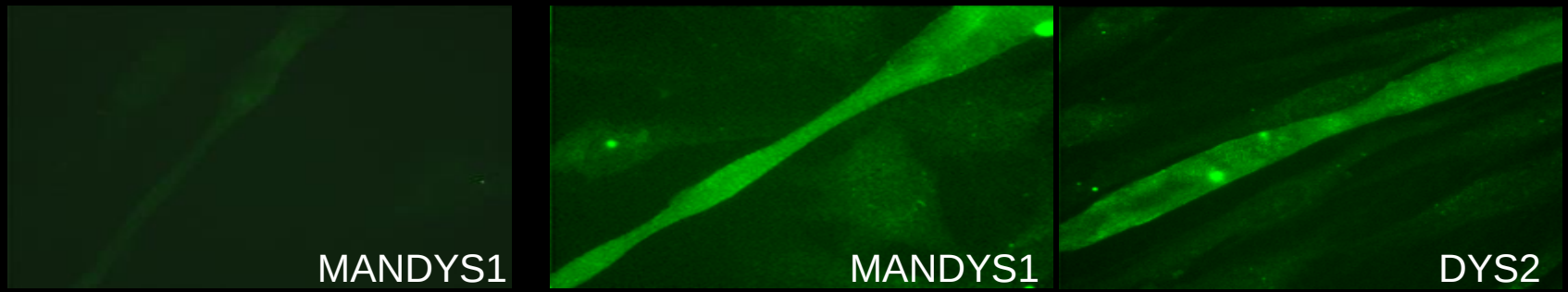


Exon 51 skipping in Δ exon 48-50 cells



NT

48 post transfection

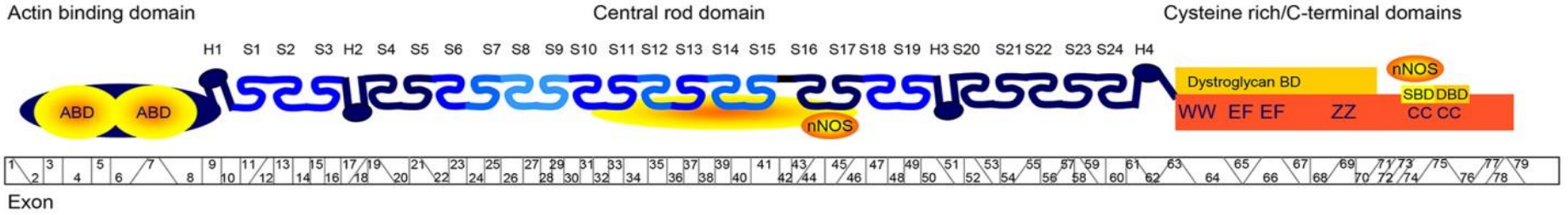


Question 2:

Q2. Which chemistry do you chose for your development (20 years ago)?

- The standard chemistry for which PK/PD and safety data is available from applications for other diseases
- A more recently developed chemistry with better efficiency in cultured cells, but for which PK/PD and safety data are not yet available

Mutation specific approach



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%

Communication

- Not applicable to all patients (mutation specific)
- Patient education
- Explain how approach works
  - www.exonskipping.nl
  - www.dmd.nl/gt/dance
- **Realistic expectations**
- **Slows down disease progresion**
- **Not a cure**

Question 3:

Q3. Do you think patients want a slower disease progression?

- No, this prolongs their suffering
- Yes, but only if ambulation can be maintained/prolonged
- Yes, but only if it increases survival
- Yes, but only if there are no side effects
- Yes, they will want a slower progression even if this means side effects

Is this what patients want?

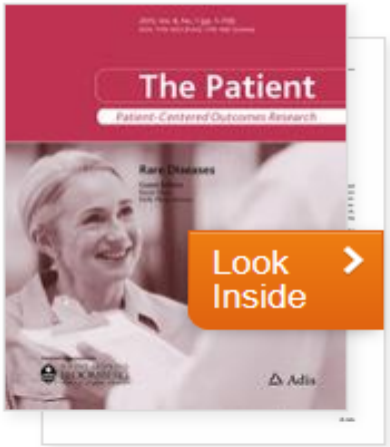
Parent Project Muscular Dystrophy
LEADING THE FIGHT TO END DUCHENNE

Leading Article
The Patient - Patient-Centered Outcomes Research
February 2015, Volume 8, Issue 1, pp 19-27

First online: 19 December 2014

Caregiver Preferences for Emerging Duchenne Muscular Dystrophy Treatments: A Comparison of Best-Worst Scaling and Conjoint Analysis

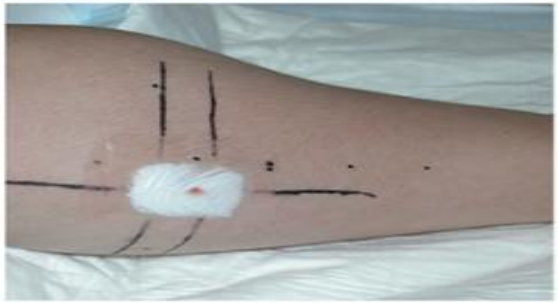
Ilene L. Hollin, Holly L. Peay, John F. P. Bridges

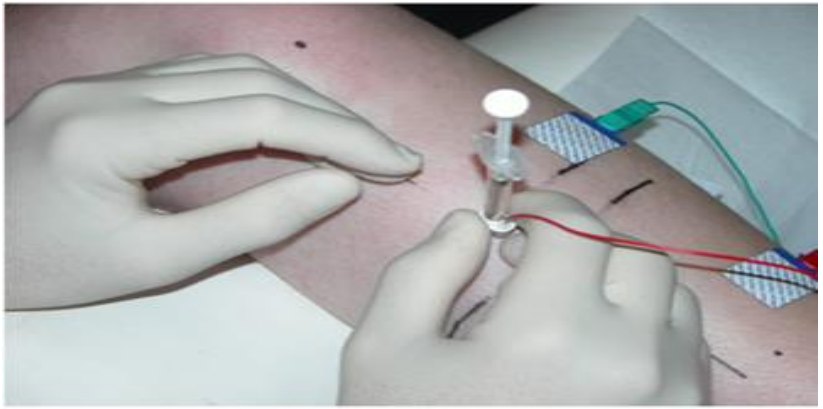


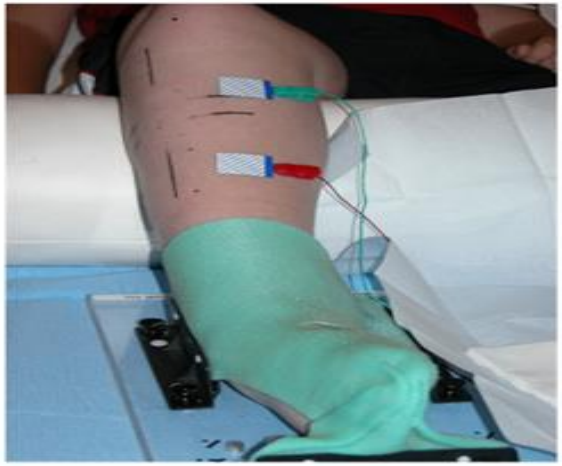
Article Metrics

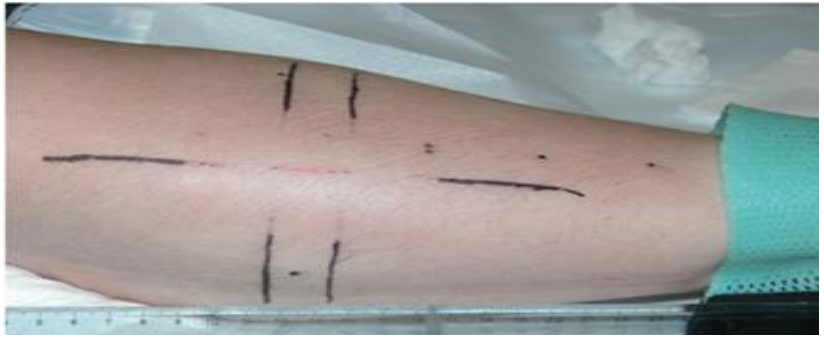
Clinical development

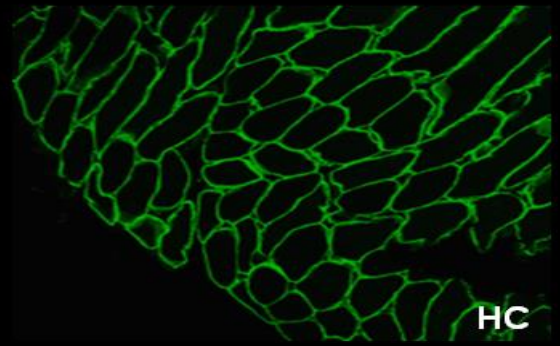
Clinical development

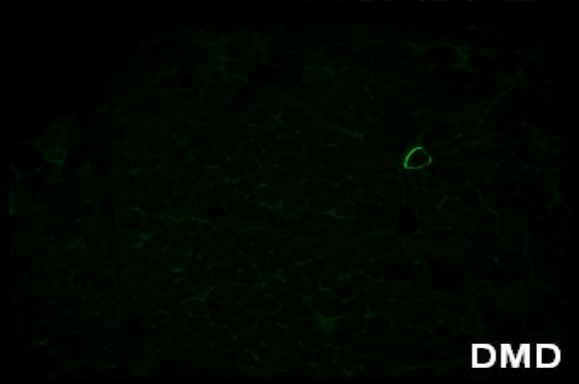









HC


DMD

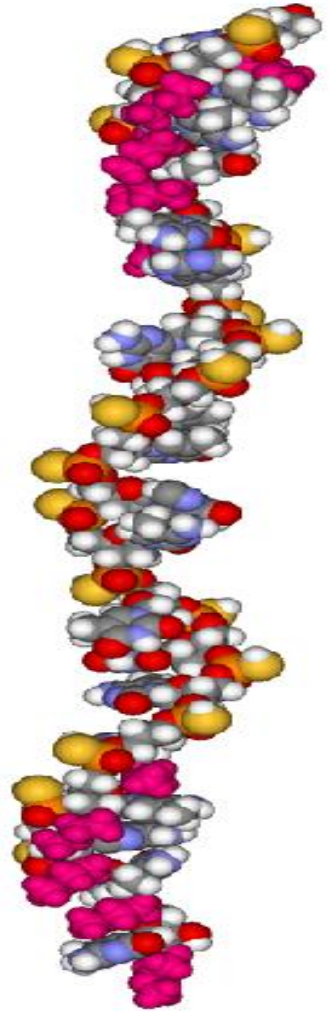
Question 4:

Q4. How many muscles are there in a human body?

- <100
- 100-200
- 200-300
- 300-400
- 400-500
- >500

Considerations for systemic delivery

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

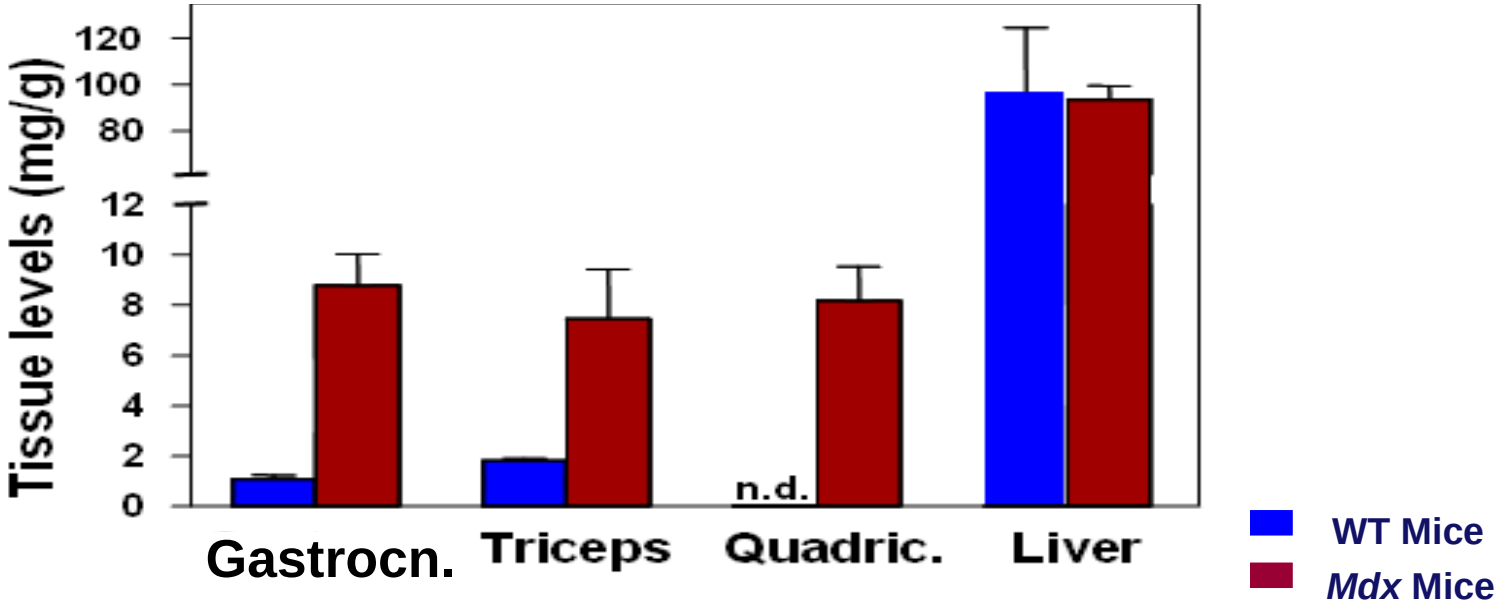


Mdx mouse model

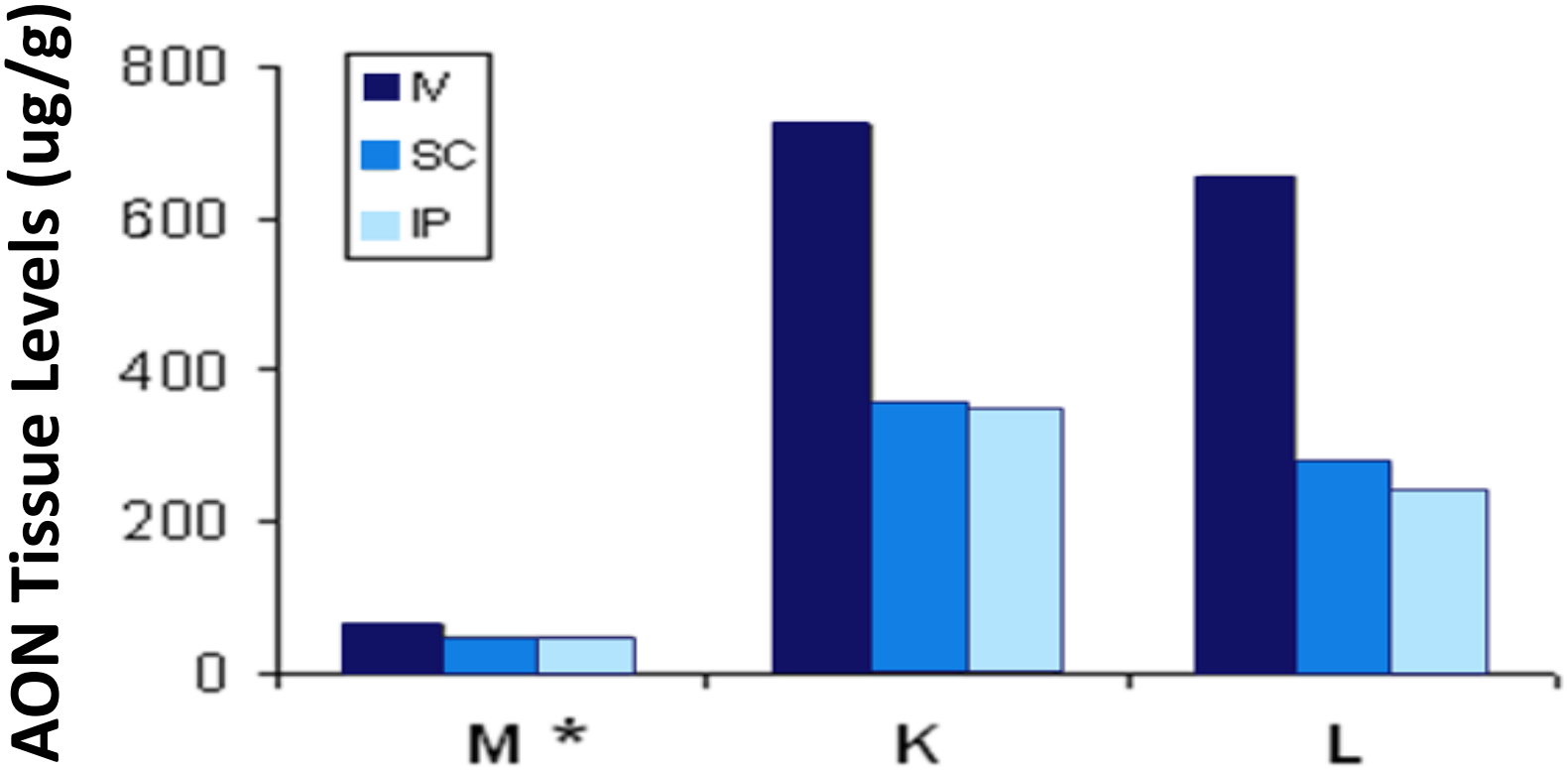
- Spontaneous nonsense mutation in exon 23
- No dystrophin production
- Dystrophic muscles
- Milder phenotype
- Needs exon 23 skipping

Systemic studies in *mdx* mice

AON levels in muscle and Liver



Systemic studies in *mdx* mice



Question 5:

Q5. Which route of administration do you select for clinical development?

- IV

  + Higher amounts of AON are delivered to muscle

  - Higher amounts of AON end up in liver and kidney

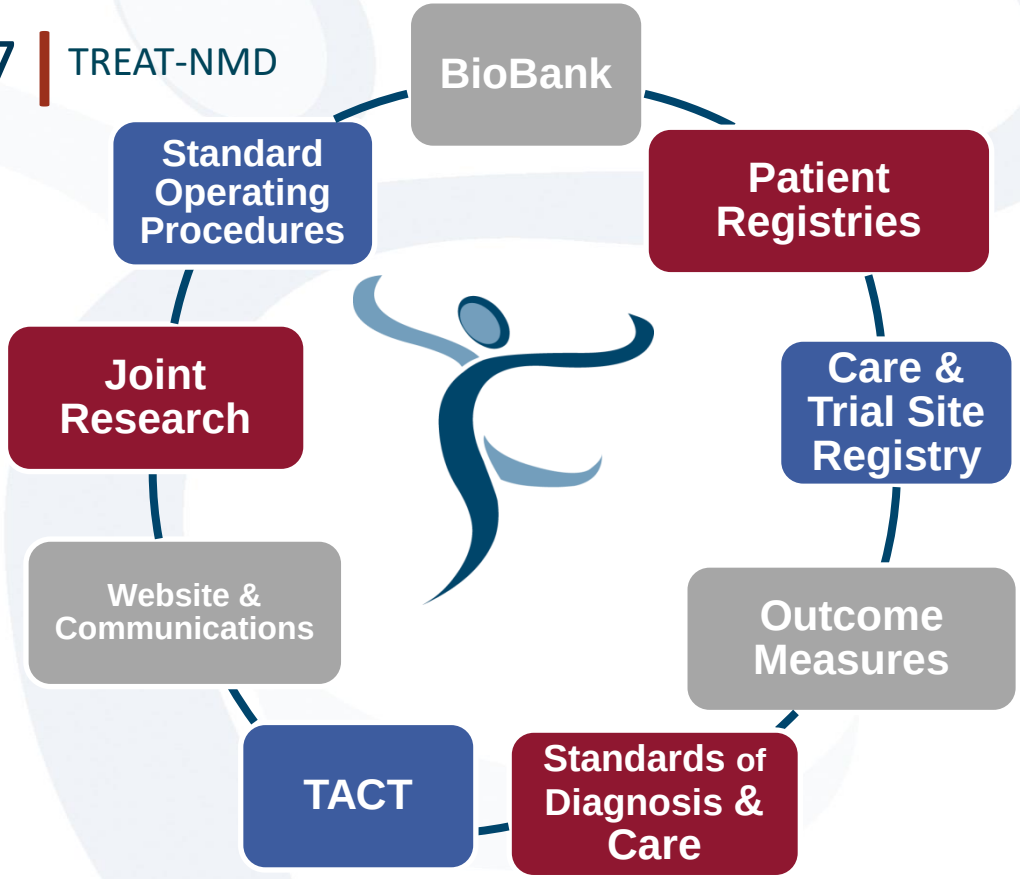
  - IV delivery requires hospital visit

- SC

  - Lower amounts of AONs are delivered to muscle

  + Lower amounts of AON end up in liver and kidney

  - Home delivery may be possible in the future



TREAT-NMD Neuromuscular Network

2007-2011

EU funded Network

2012 onwards

Alliance funded through multiple streams with global partners & membership

Governance

Executive Committee

Current Chair: Jim Dowling

www.treat-nmd.eu

Question 6:

Q6. What do you do?

- Postpone trials until the DMD field is clinical trial ready
- See whether outcome measures are available in other fields that can be borrowed

Trials were initiated (2008-2010)

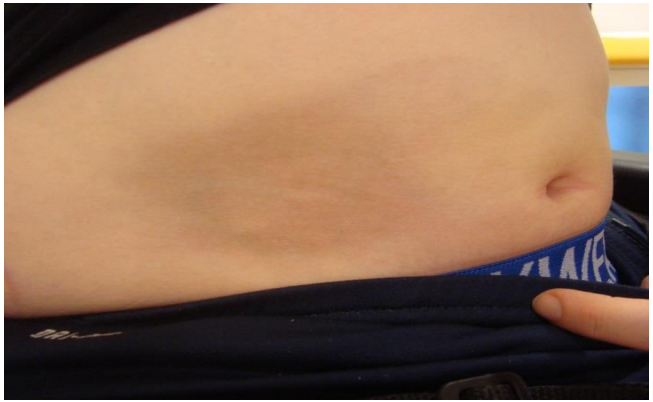
Prosensa → Lisenced exon 51 skipping drug to GSK

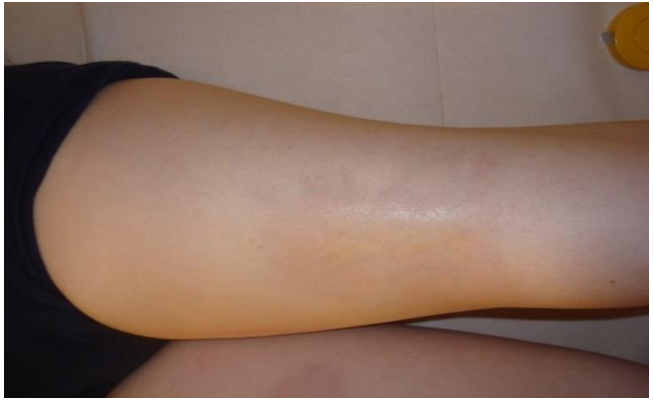
- Subcutaneous delivery
- 2a: Dose escalation (n=12)
- 2b: Dose regimen (n=51)
- 2b: Dose comparison (n=51)
- 3: Efficacy study (n=186)
- Open label extension study for each
- Primary endpoint: 6 minute walk test

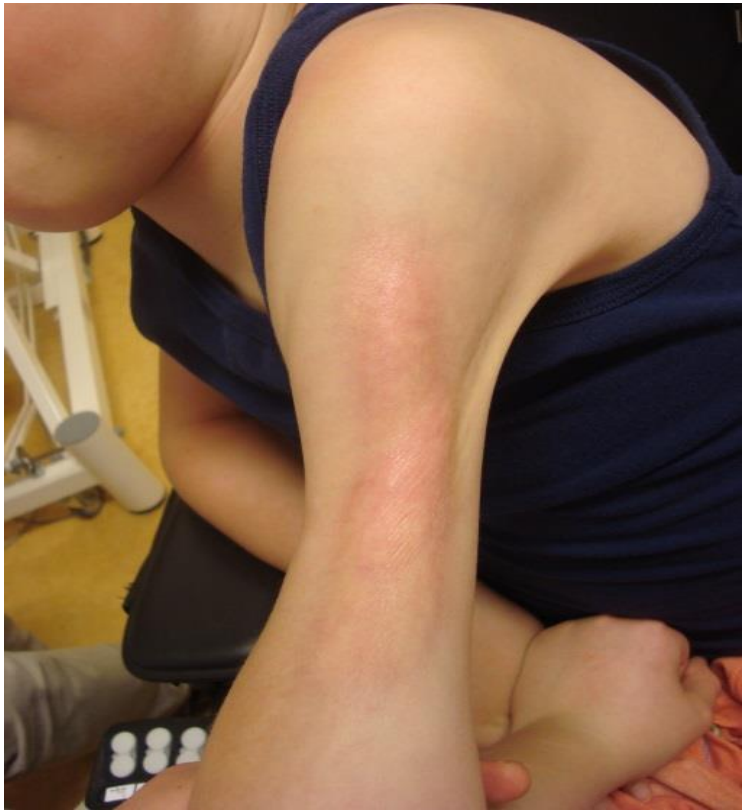
Side effects observed

- Local injection reactions
  - Known effect of subcutaneous delivery of PS AONs
  - Intraveneous delivery: no injection reactions
- Proteinuria (reversible during treatment breaks)
- Thrombocytopenia in some patients

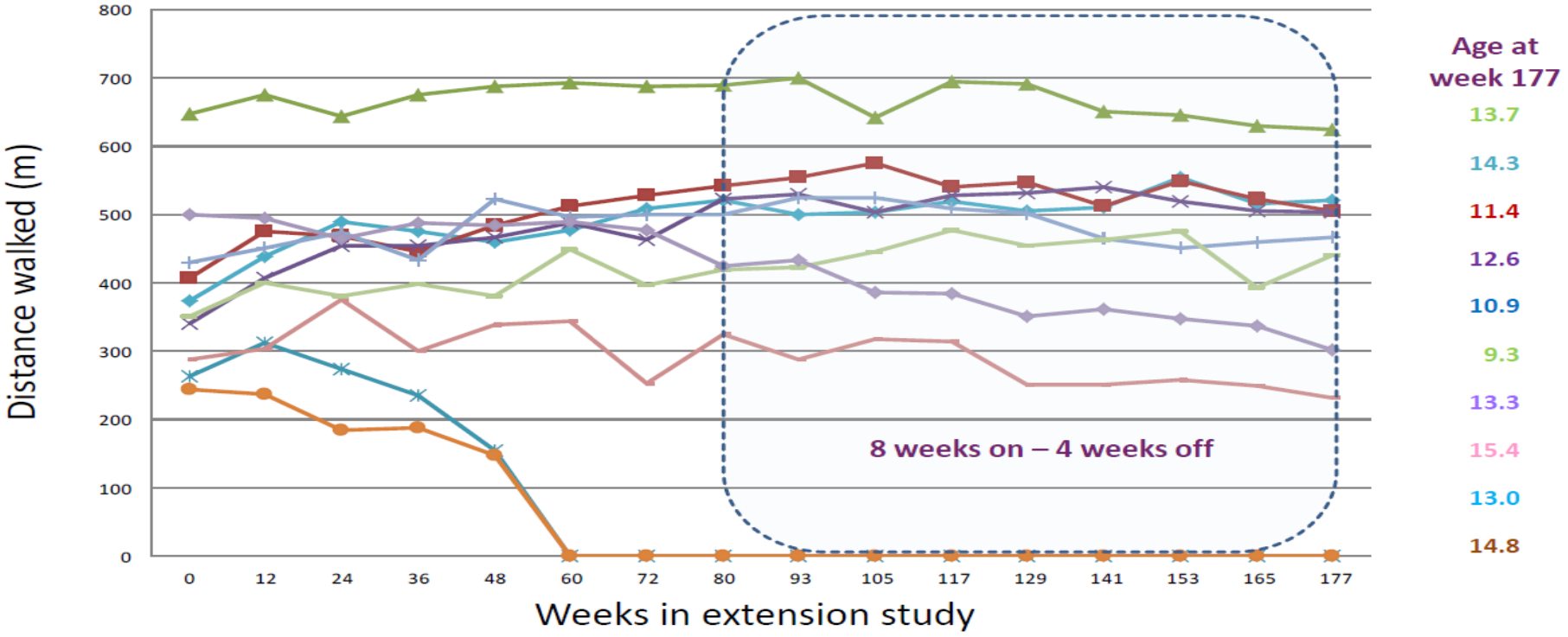
Drisapersen - skin reactions







Open label study after dose escalation



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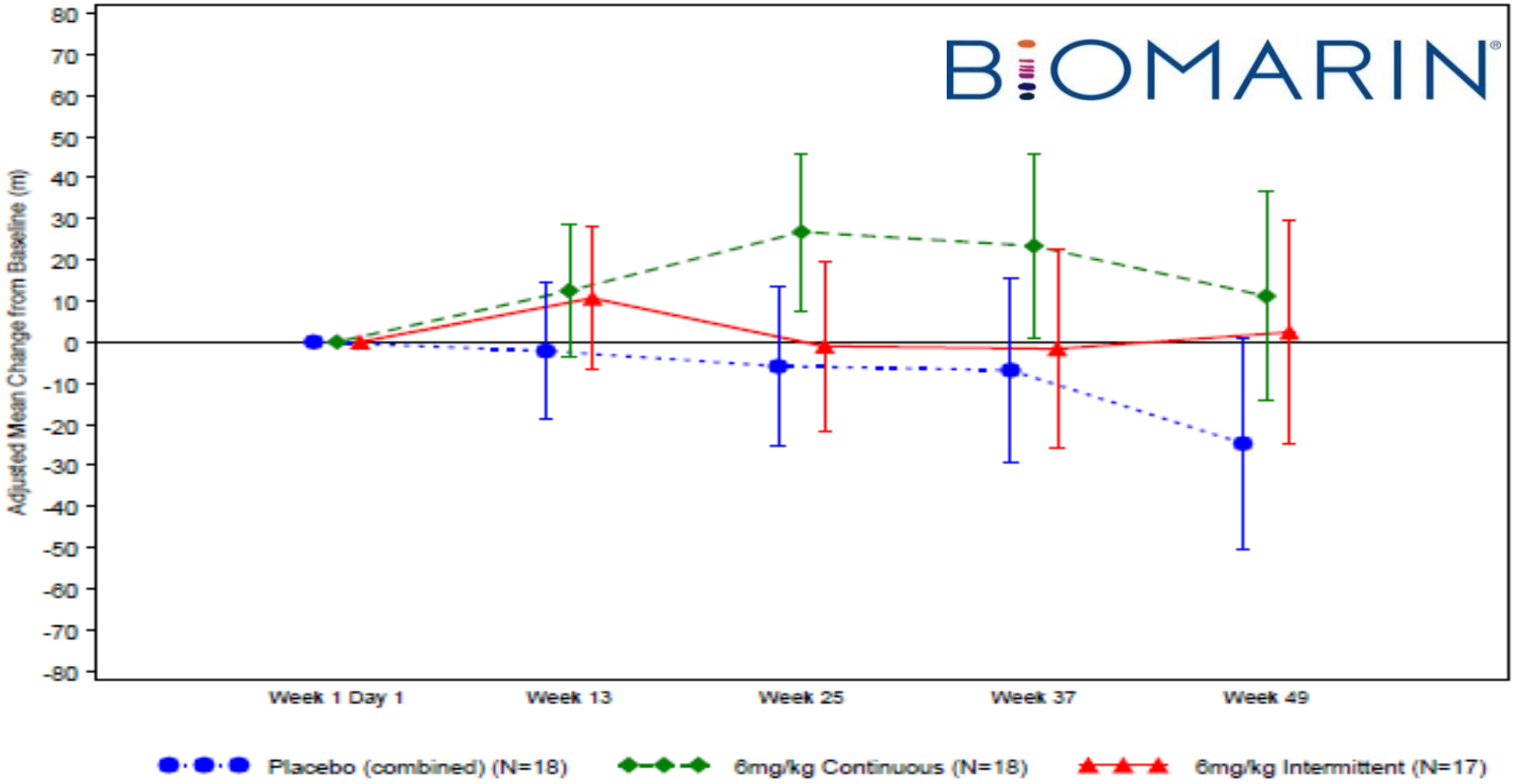
Q7. Do you think this drug works?

- No
- Yes
- I need more information
- I do not know

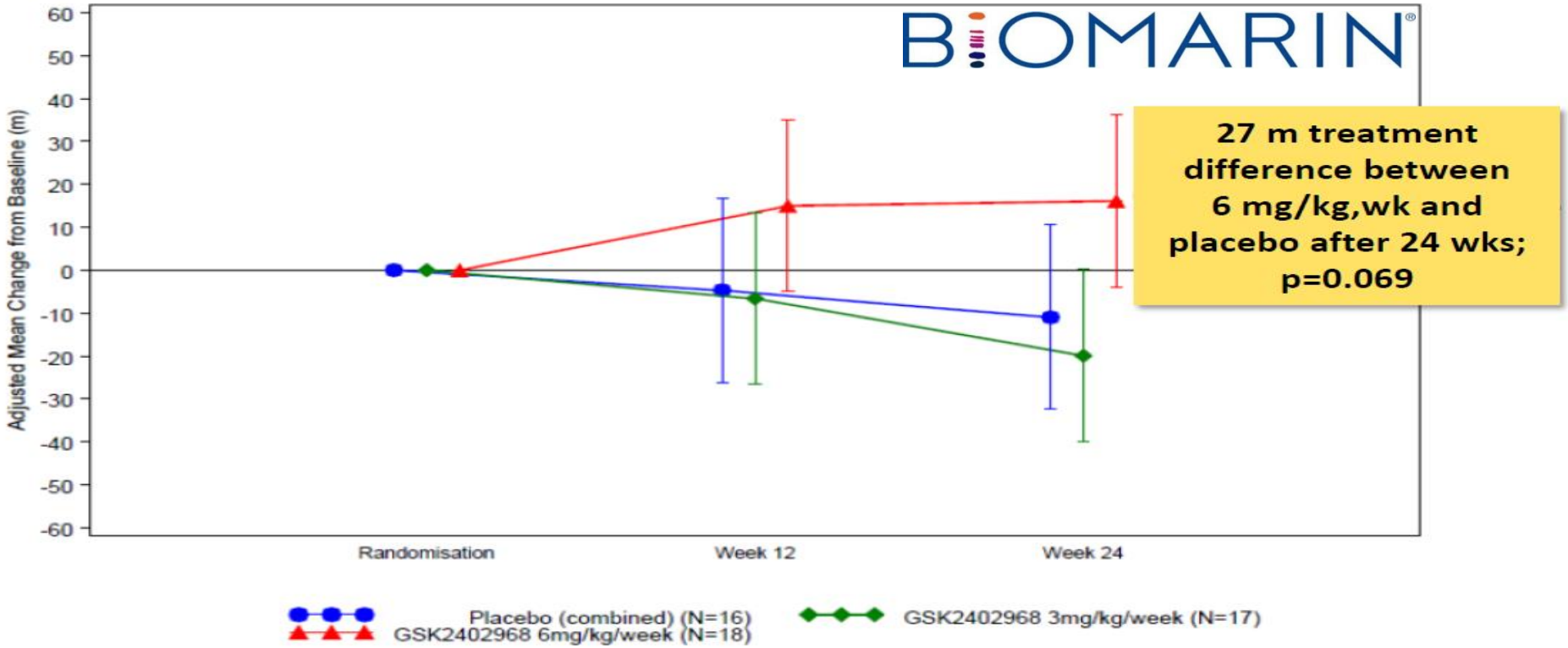
Phase 2b. Dose regimen study



Q8. Do you think this drug works?

- No
- Yes
- I need more information
- I do not know

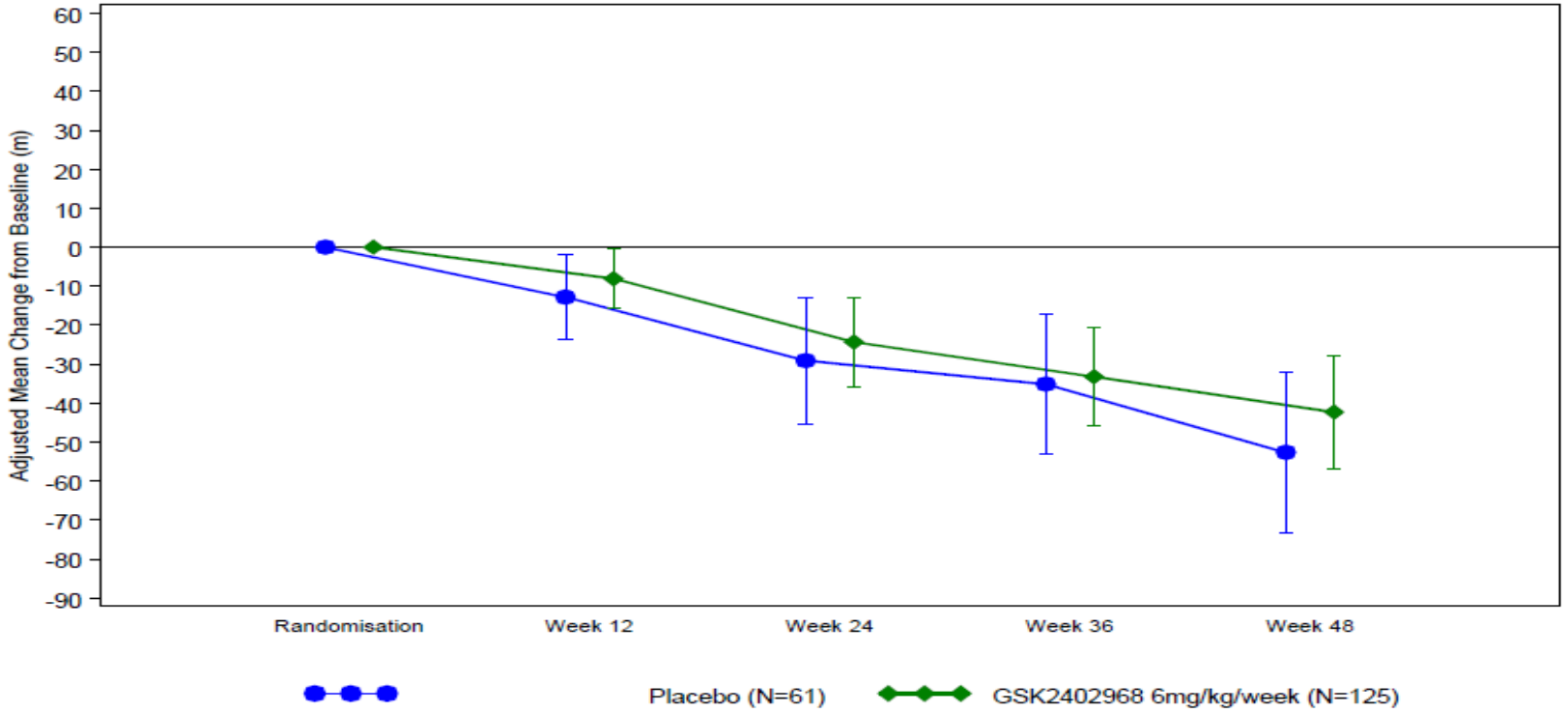
Phase 2b. Dose comparison



Q9. Do you think this drug works?

- No
- Yes
- I need more information
- I do not know

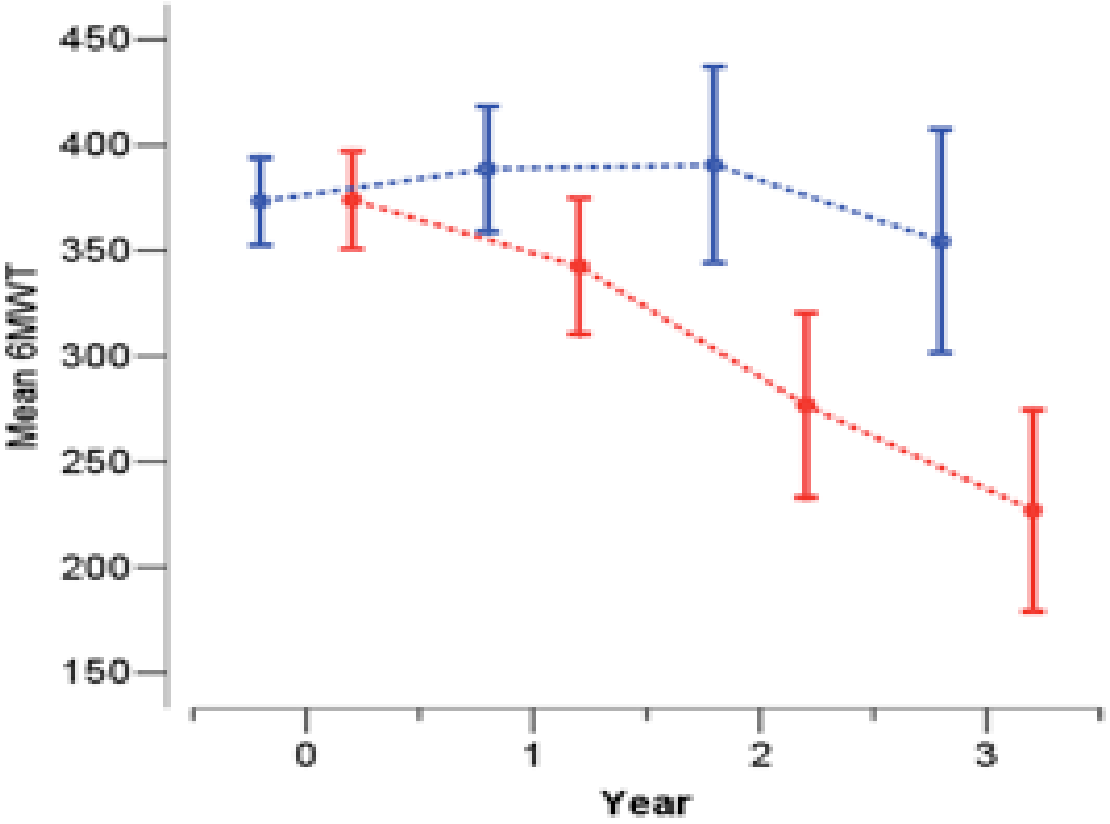
Phase 3. Efficacy study



Q10. Do you think this drug works?

- No
- Yes
- I need more information
- I do not know

What we know now



Blue: below 7

Red: above 7

Q11. Do you think this drug works?

- No
- Yes
- I need more information
- I do not know

What happened then

- GSK dropped drisapersen
- Prosensa acquired by BioMarin → additional analyses
- Phase 3 population more advanced disease
  - Younger subgroup shows response
  - Older patients show response after longer treatment (OLE)
- Applied for approval with FDA and EMA
- Approval denied by FDA
- Application withdrawn from EMA

And then…

BIOMARIN®

May 31, 2016

Previous Release | Next Release

BioMarin Announces Withdrawal of Market Authorization Application for Kyndrisa™ (drisapersen) in Europe

Lessons learned

- Interaction with regulators started too late
- Lack of outcome measures & natural history data
- Suboptimal trial design

Now

- Ongoing dialogue academics, patients and regulators in EU
- Development new outcome measures
- Future trials will be better

DMD exon skipping state of the art

- 2OMePS AON development stopped (drisapersen)
- 4 AONs approved (USA and Japan, none in Europe)
- PMO (so no PS backbone)
- Exon 51, exon 45 and exon 53 (2)
- Based only on dystrophin restoration (<1-5%)
- Functional effects still have to be confirmed
- Clinical trials ongoing

Q12. Do you think ~1% of dystrophin is enough for slower disease progression??

- No
- Yes
- I need more information
- I do not know

Room for improvement

Focus areas:

1. Improve DMD exon skipping
2. Improve model systems and natural history
3. Improve muscle quality
4. Validation lab
5. Biomarkers for DMD
6. Study dystrophin transcript processing
7. Dystrophin and brain
8. Exon skipping treatment for very rare mutations (DCRT)
9. Patient education
10. Collaborations on exon skipping for other genes/diseases

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