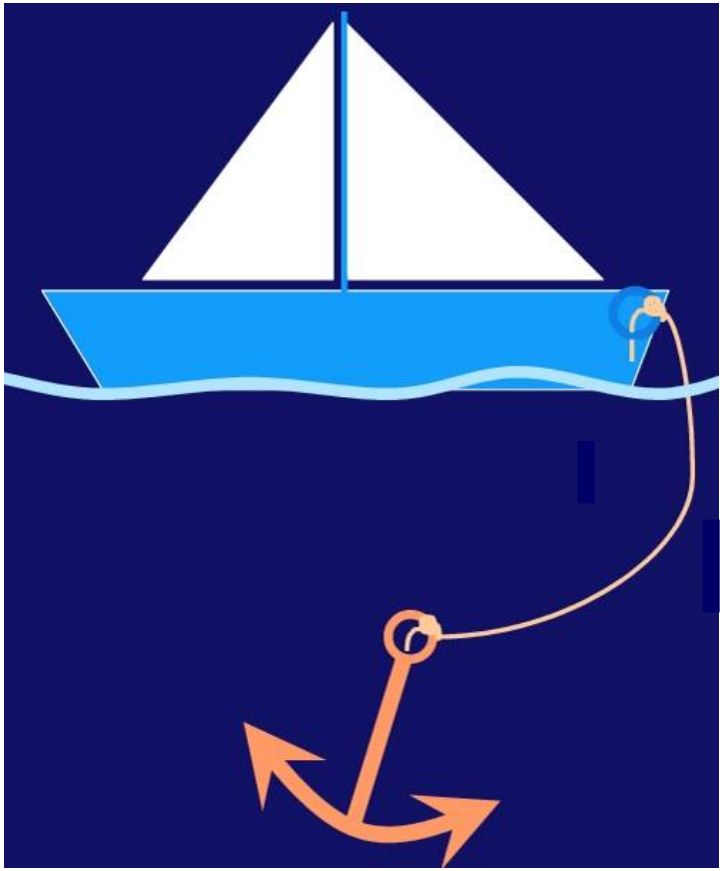
LEIDEN UNIVERSITY MEDICAL CENTER

Future directions for exon skipping

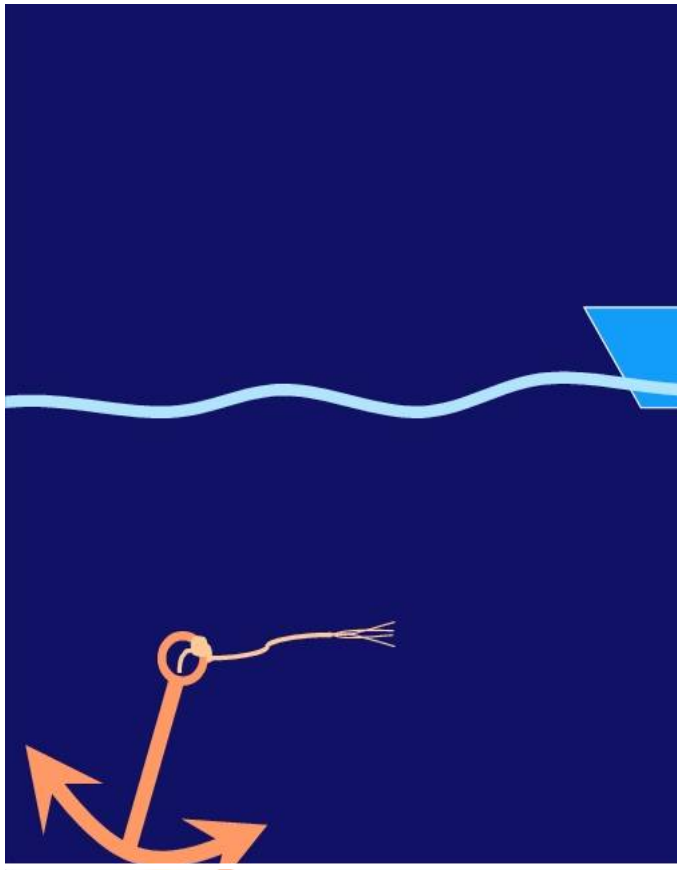
12 January 2014

Exon skipping

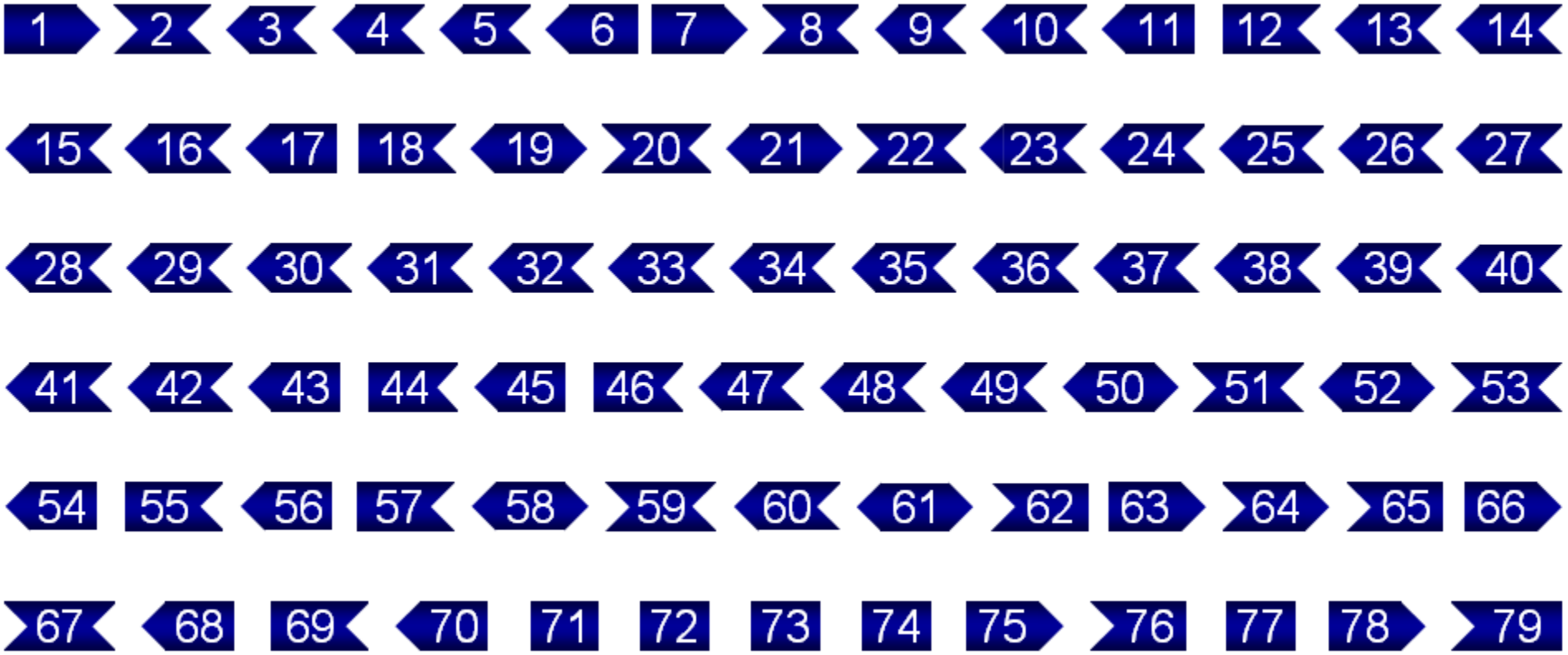
Normal



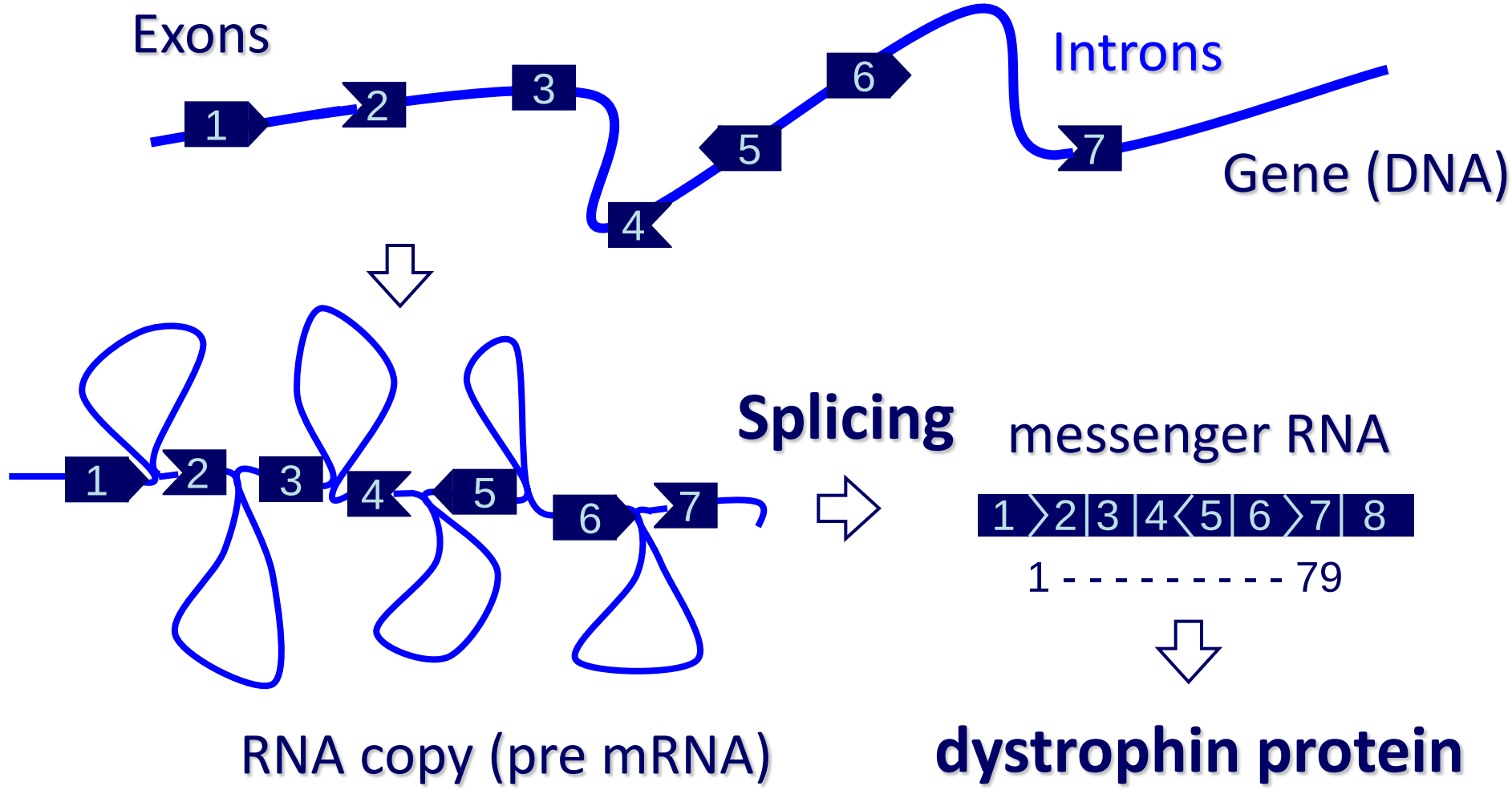
Duchenne



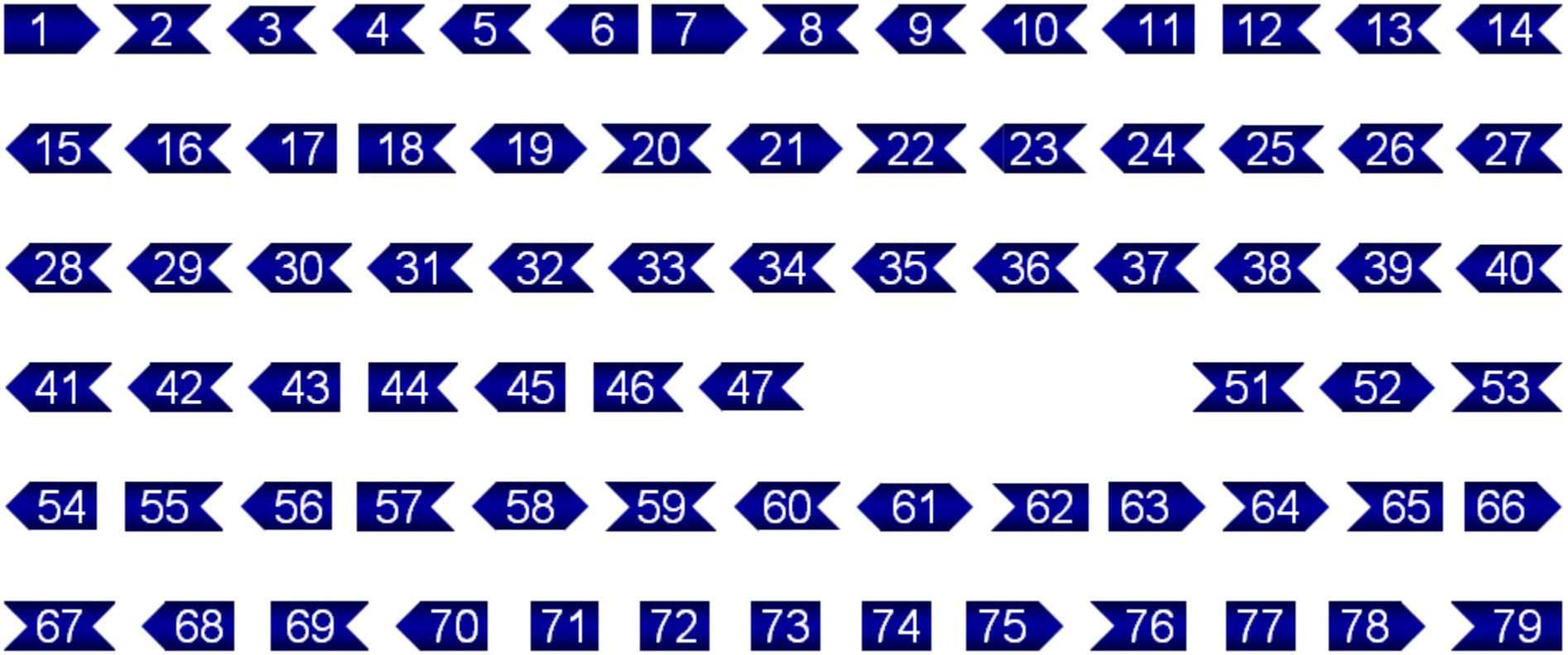
Dystrophin gene



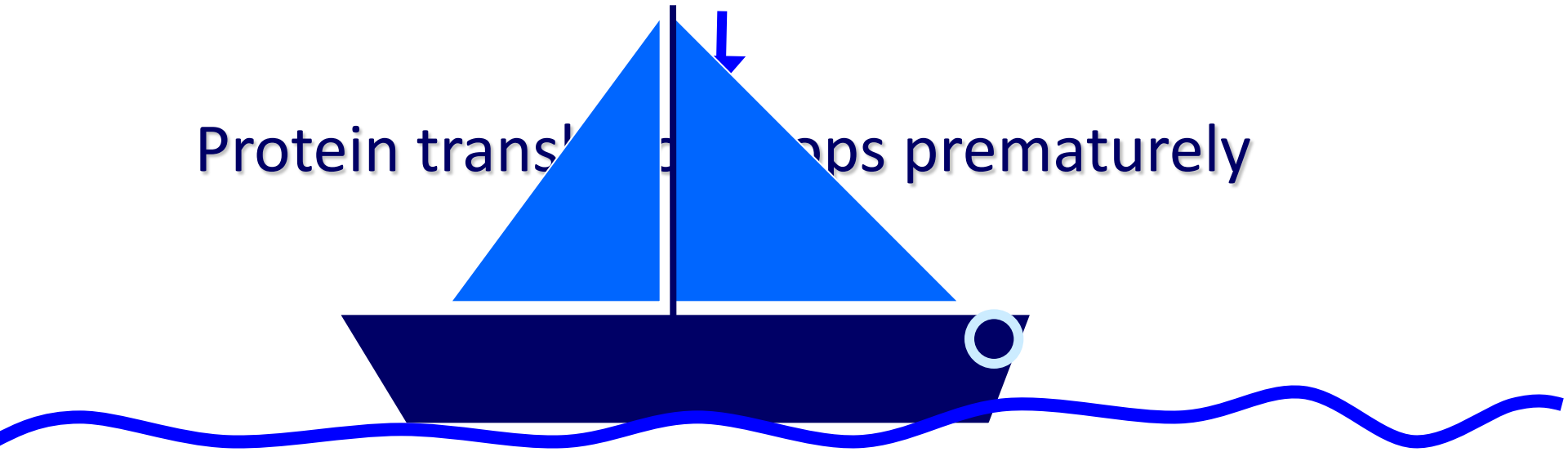
Splicing

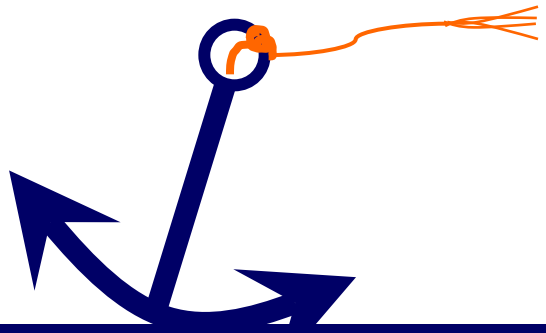

Exons
1
2
3
4
5
6
7
Introns
Gene (DNA)
Splicing
messenger RNA
1 2 3 4 5 6 7 8
1 - - - - - - - - - 79
RNA copy (pre mRNA)
dystrophin protein

Duchenne: genetic code disrupted



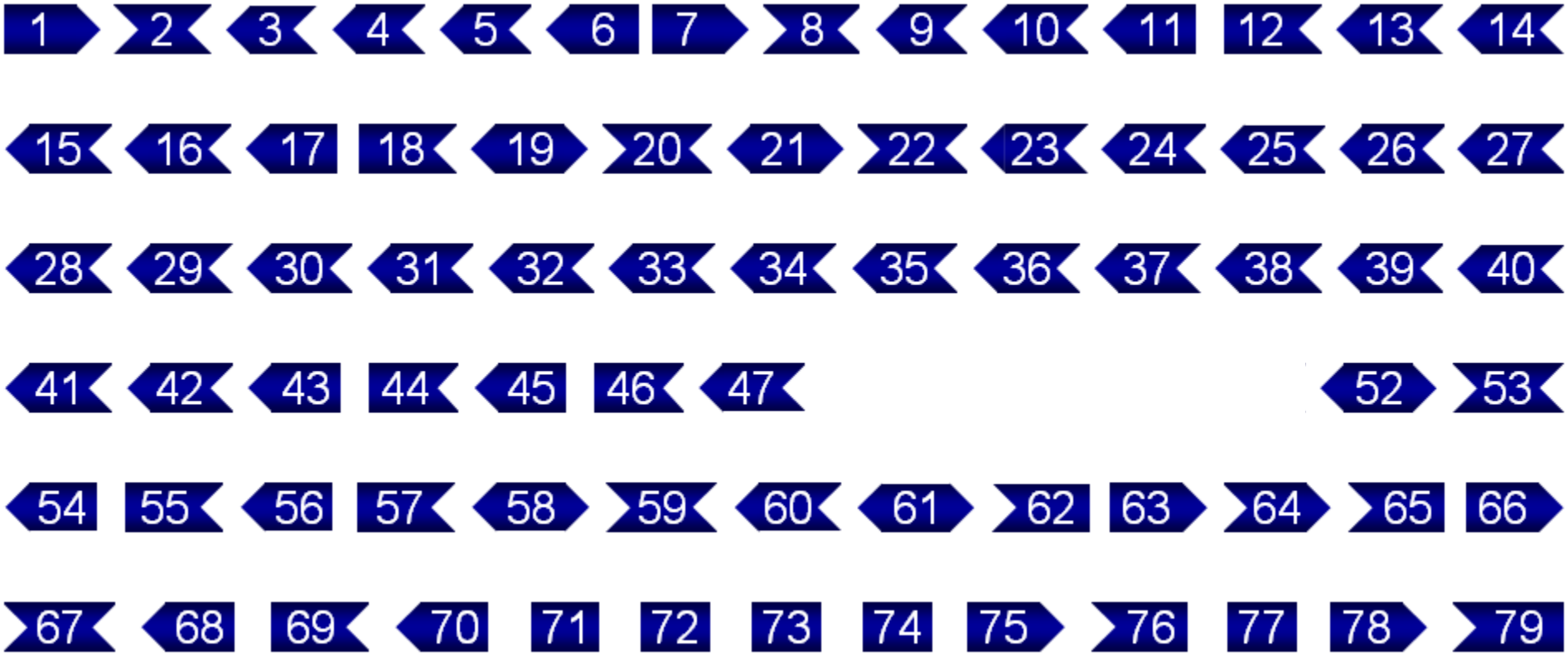
Duchenne: genetic code disrupted



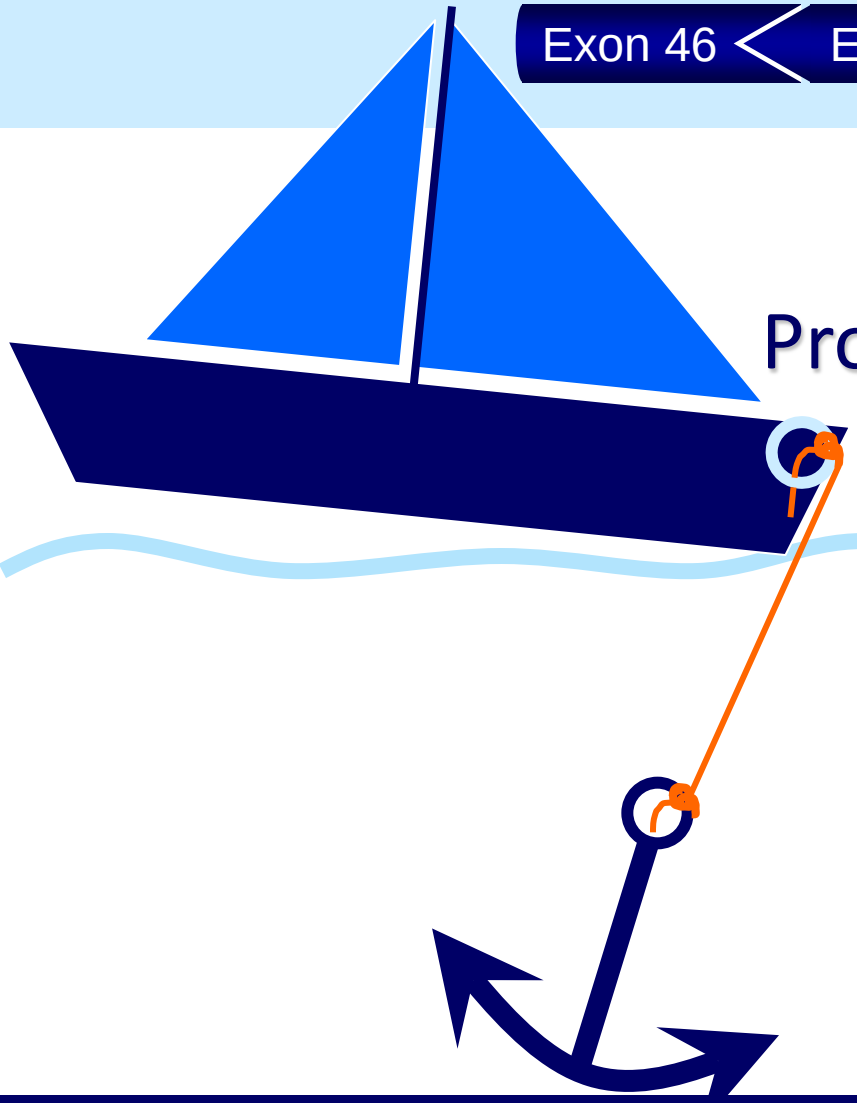


Dystrophin not functional

Becker: genetic code maintained



Becker: genetic code maintained

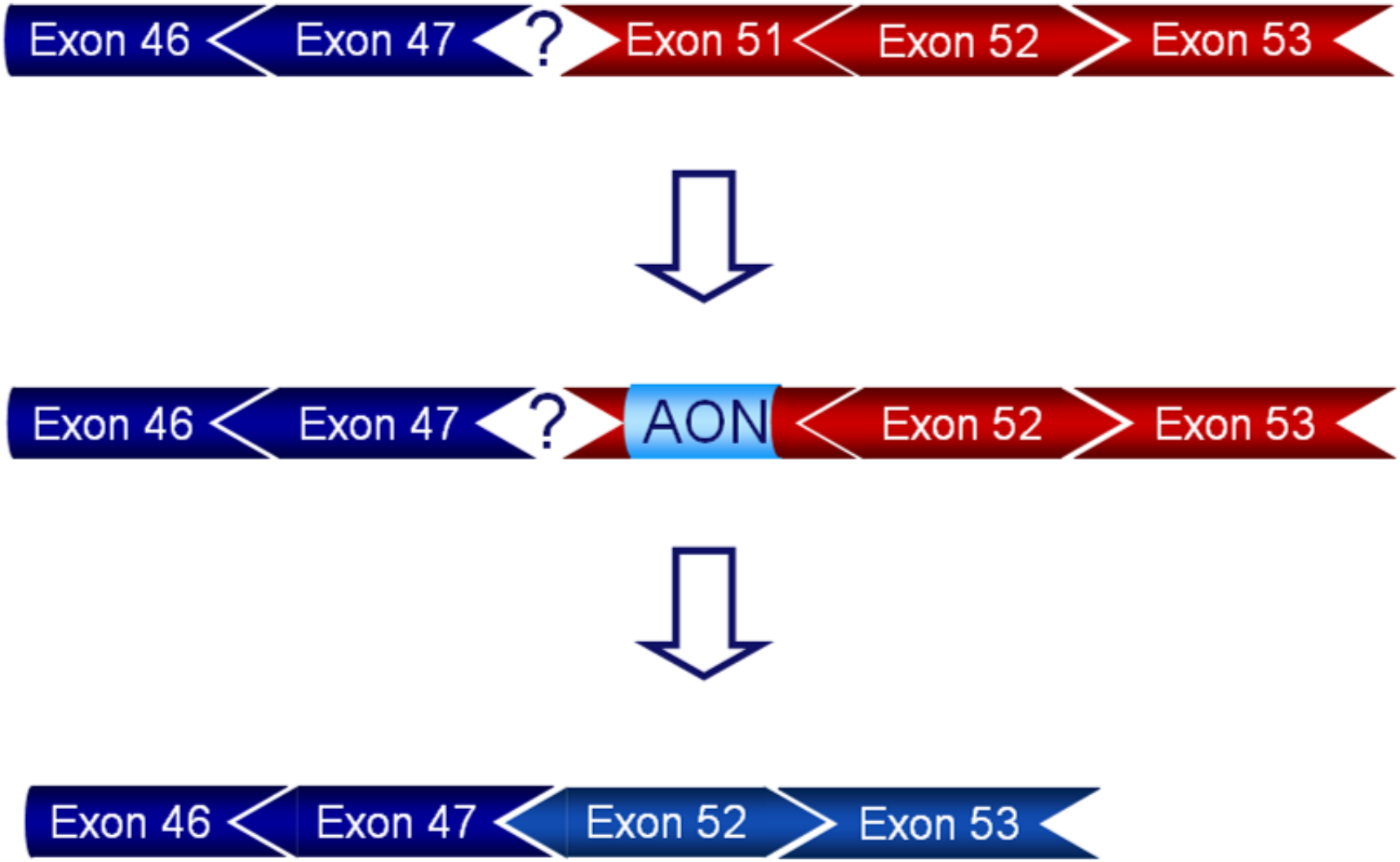


Exon 46 | Exon 47 | Exon 52 | Exon 53

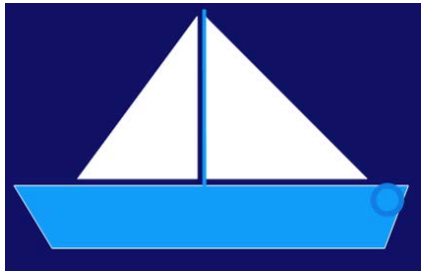
Protein translation continues

Dystrophin partly functional
Less damage

Exon skipping: restore genetic code



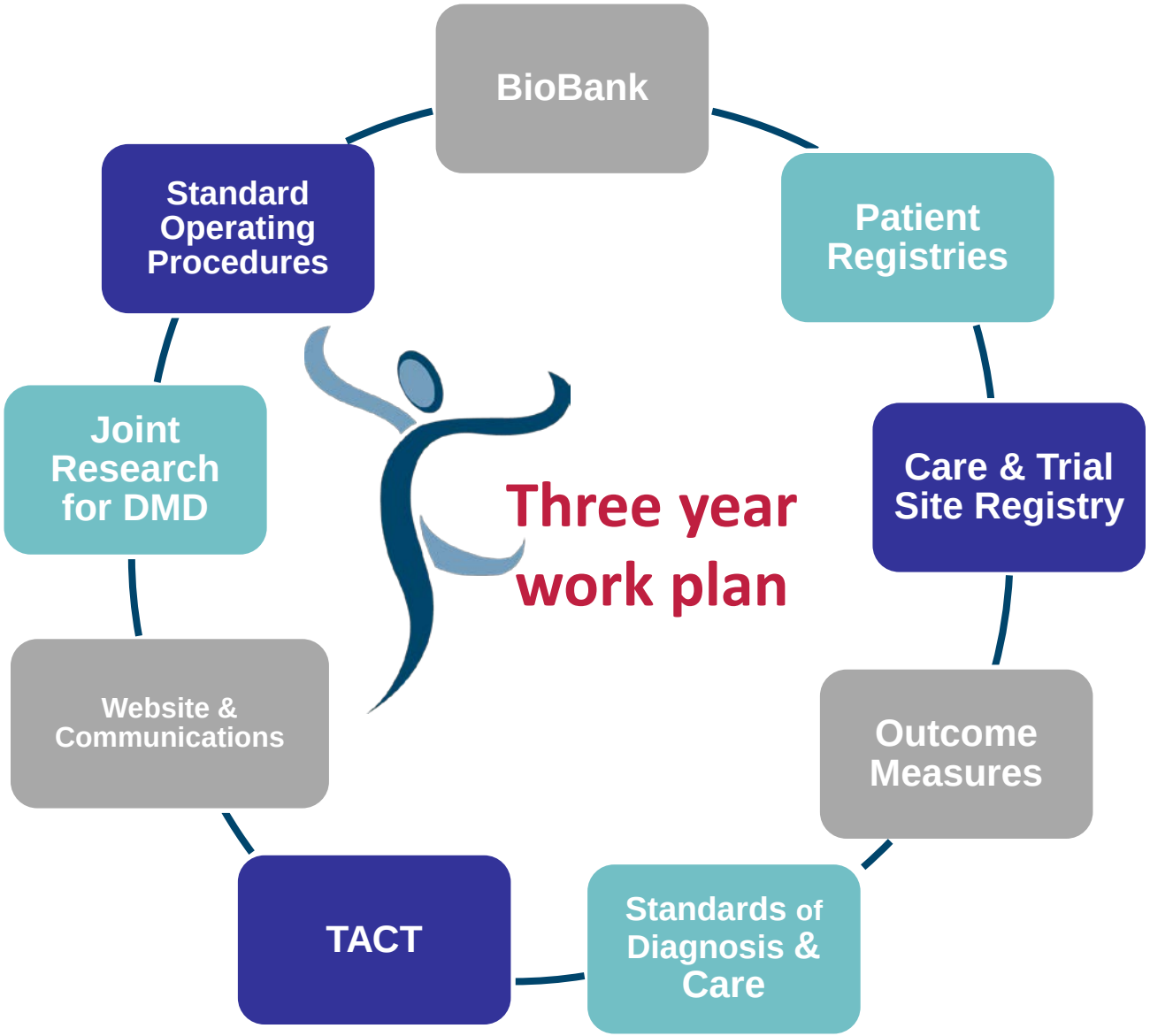
Applicability



hotspot

Exon	All mutations	Deletions	Duplications	Small mutations
51	13.0%	19.1%	0.3%	3.0%
45	8.1%	11.8%	0.2%	2.2%
53	7.7%	11.4%	0.1%	1.5%
44	6.2%	8.85	0.4%	2.7%
46	4.3%	6.2%	0.2%	1.6%
52	4.1%	5.7%	0.5%	2.3%
50	4.0%	5.6%	0.2%	1.9%

Aartsma-Rus Hum Mutat 2009, 30:293-9



2007-2011

EU funded Network

2012 onwards

Alliance funded through multiple streams with global partners & membership

Governance

Chair – Hanns Lochmüller
Vice – Annemieke Aartsma-Rus

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

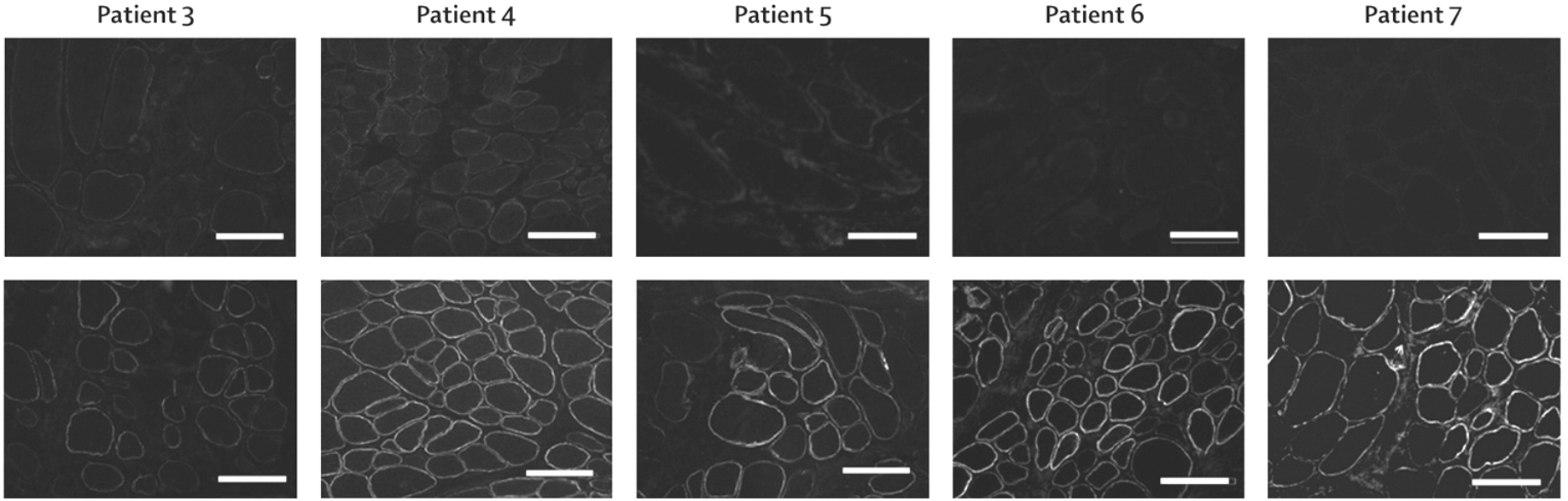
www.treat-nmd.eu

Exon skip chemistries

- Two chemistries in clinical development
- GSK/Prosensa: 2’-O-methyl phosphorothioate (drisapirsen)
- AVI-Biopharma/Sarepta: phosphorodiamidate morpholino oligomers (eteplirsen)
- Exon 51

Eteplirsen: Intramuscular trial (AVI)

- Two doses tested (0.09 and 0.9 mg)
- EDB muscle
- Exon skip in all doses
- Dystrophin restoration only for high dose



Kinali Lancet Neurol 2009, 8: 918-28

Systemic study Sarepta

Eteplirsen (0.5, 1, 2, 4, 10 & 20 mg/kg)

- Intravenous delivery
- Weekly for 12 weeks
- 2-4 patients per group (19 total)
- Biopsies
  - Pre and 2 weeks post treatment
  - Dystrophin in 7/19 patients
  - 3 good responders
  - No functional effects observed

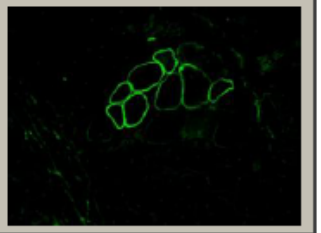
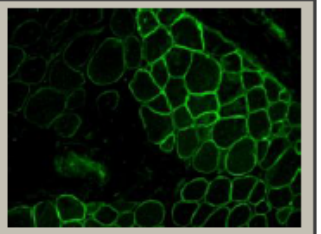
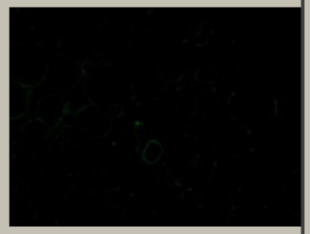
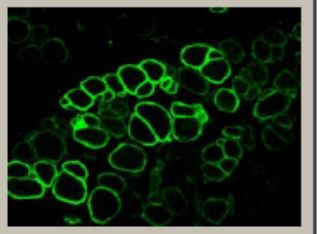
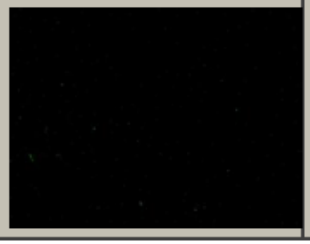
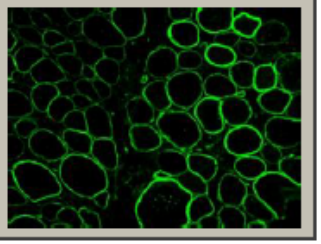
Cirak et al, Lancet 2011, 378: 595-605

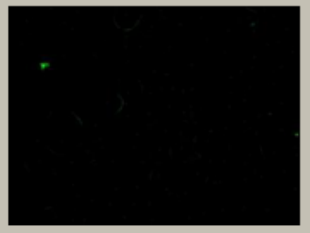
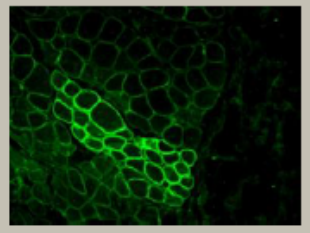
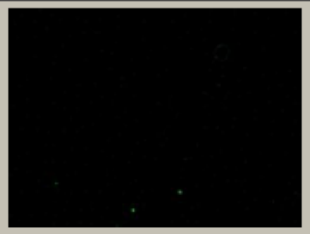
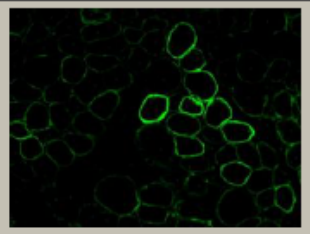
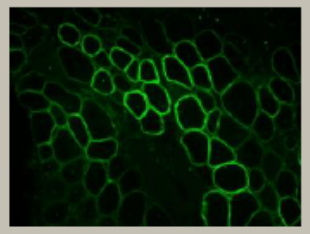
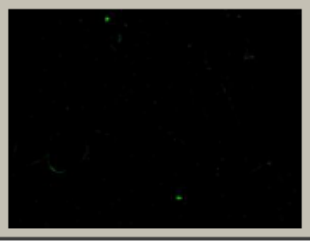
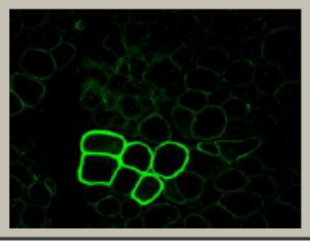
Systemic study Sarepta II

AVI-4658 (30 and 50 mg/kg)

- Intravenous delivery
- Weekly 12 weeks 50 mg/kg: biopsy
- Weekly 24 weeks 30 mg/kg: biopsy
- Weekly placebo 12 or 24 weeks: biopsy
- After 24 weeks treatment: all 30 or 50 mg/kg/week
- Patients have been treated for >90 weeks now

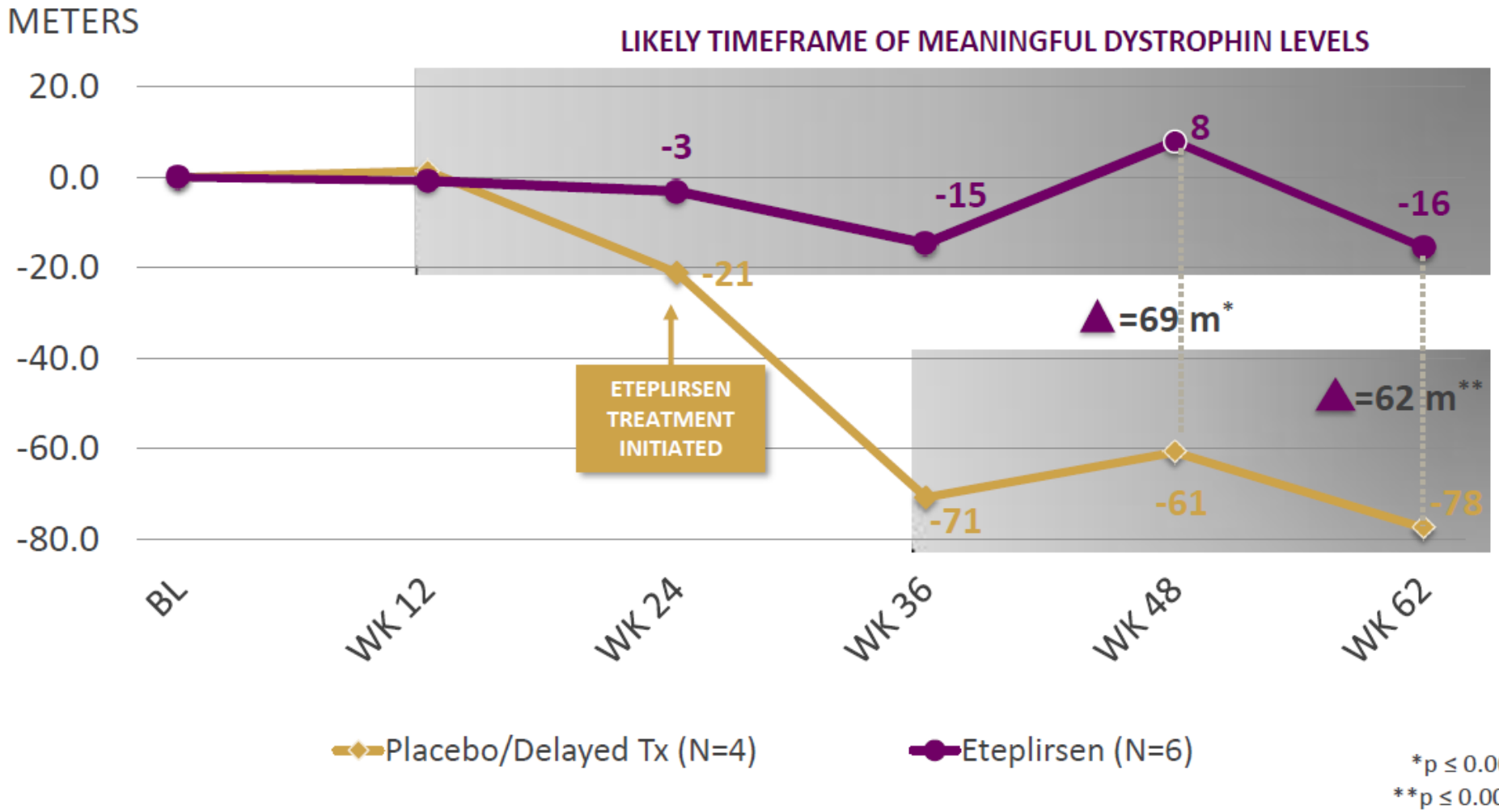
Dystrophin staining

50 MG/KG		
Patient	Pre-Tx	48 wks of Tx
03		
04		
12		
15		

PLACEBO/DELAYED TX		
Patient	Pre-Tx	24 wks of Tx
05 (50 mg/kg)		
13 (50 mg/kg)		
08 (30 mg/kg)		
07 (30 mg/kg)		

6MWT CHANGE FROM BASELINE TO WEEK 62[1]:

DATA BASED ON MAXIMUM 6MWT SCORE WHEN TEST WAS REPEATED



*p ≤ 0.003
**p ≤ 0.007

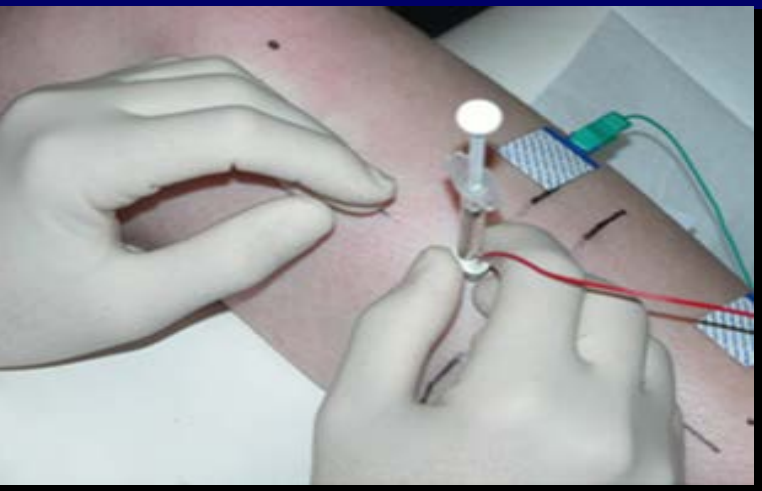
Note: Statistical analysis based on using MMRM test.

[1]Modified Intent –to-Treat (mITT) population

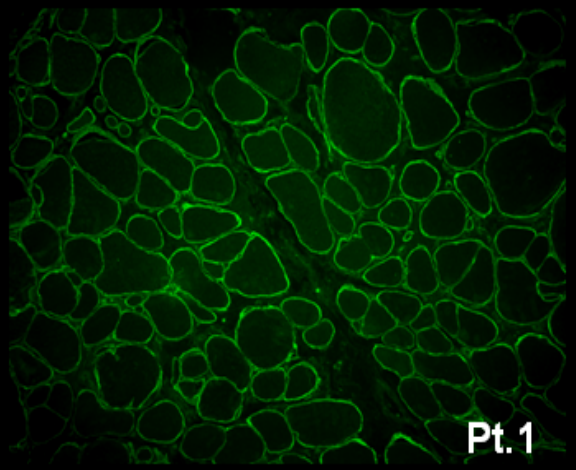
Eteplirsen summary

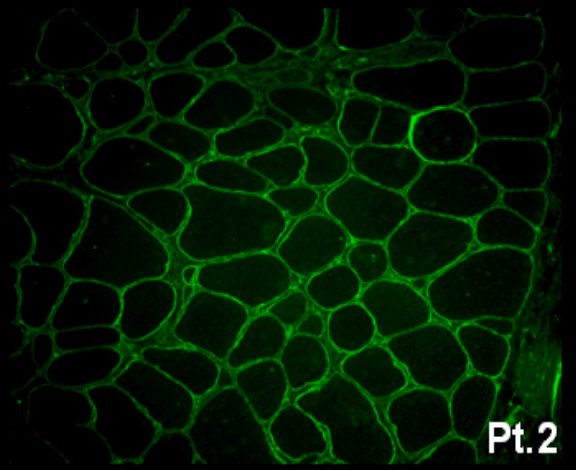
- Dystrophin restored for all patients 24 weeks after treatment
- 6 minute walk test stabilized in 10/12 treated patients
- No real placebo group
- Sarepta plans confirmatory study for 2014
- Also preclinical work ongoing for exon 50, 45, 53 and 44)

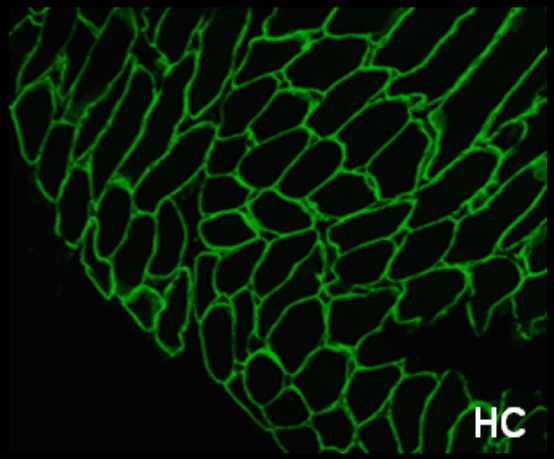
Dirsapersen Intramuscular trial

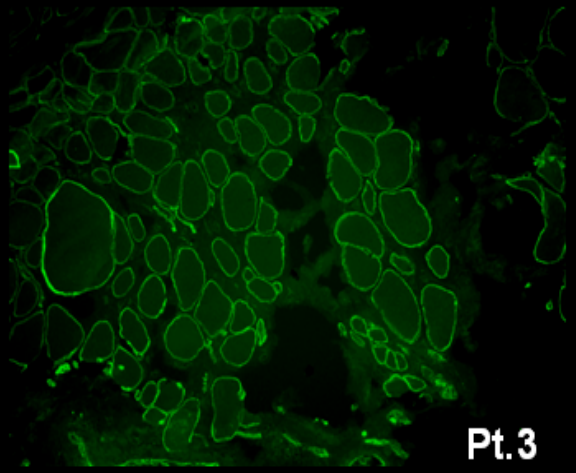


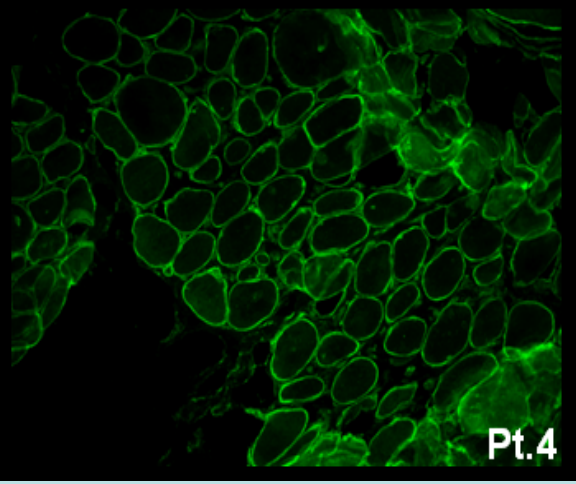
0.8 mg in TA











Annemieke Aartsma-Rus

Van Deutekom NEJM 2007; 357: 2677-86

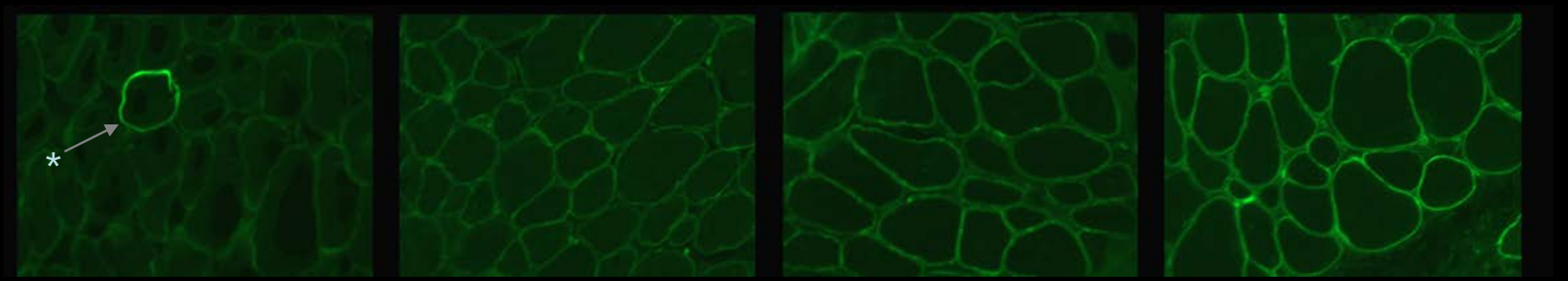
Systemic trial (GSK/Prosensa)

- Subcutaneous treatment for 5 weeks
  - 0.5, 1, 2 & 6 mg/kg
- Dystrophin restored in 10/12 patients
- 60-100% fibers dystrophin positive
- Dystrophin levels up to 15.6%
- Dose dependent increase in dystrophin levels

Systemic study (GSK/Prosensa)

Not treated

Dose



PROSENSA

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

Systemic trial (GSK/Prosensa)

- 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
- All patients still in trial
- Treated now for almost 4 years

PROSENSA

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

Open Label Extenion Trial Drisapersen

- Side effects observed
  - Local injection site reactions
  - Proteinuria (reversible during breaks)
  - Thrombocytopenia seen for some patients
- 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)

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

➔ Increased 6 minute walk distance for patients treated with 6 mg/kg vs placebo and 3 mg/kg

Drisapersen summary

- Dystrophin restored in dose dependent manner
- 6 minute walk distance stabilized in 8/10 ambulant patients for >3.5 years
- 6 minute walk distance improved in early phase patients in 2 small placebo controlled studies
- Placebo-controlled trial in wider age range shows no significant improvement
- Further analysis ongoing in subgroups

Other trials Prosensa/GSK

Drisapersen

- Treatment drisapersen studies suspended pending further analysis

Exon 44:

- Compare subcutaneous and intravenous ongoing

Recently started: exon 45 and exon 53

Preclinical work:

- Exon 55 & exon 52 and rare mutations

Preclinical work

- Other AON chemistries
  - Tricyclo
  - Peptide conjugated AONs
  - Muscle/heart homing peptides
- Exon skipping gene therapy
  - Antisense gene in AAV
  - Longer lasting effect
  - Trial planned in France for 2014

Exon skipping summary

- Opportunities
  - AON delivery easier than genes/cells
  - Clinical trial results encouraging
- Challenges
  - Mutation specific approach
  - Need to develop many AONs to treat majority of patients
  - Repeated treatment needed (also opportunity)

COST Action BM1207

Networking towards clinical application of antisense-mediated exon skipping

Involving all key stakeholders (clinicians, scientists, industry, patients & regulators)

- Facilitate the clinical development of antisense-mediated exon skipping for rare diseases
- Exploit exon skipping for as many patients as possible

Objectives

1. Achieve consensus biochemical outcome measures
2. New regulatory models to allow testing of personalized medicine approaches
3. Foster synergistic work (workshops and key stakeholder meetings and exchange visits)
4. Address therapeutic misconception by training early stage researchers

Current parties involved

Denmark, France, Germany, Israel, Italy, Netherlands, Norway, Spain, Sweden and United Kingdom

If you are interested to join, please contact the Chair of this Action

Annemieke Aartsma-Rus, a.m.rus@lumc.nl