LEIDEN UNIVERSITY MEDICAL CENTER

Overview of therapeutic approaches

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Duchenne muscular dystrophy

Cause: no functional dystrophin protein

Consequence: Duchenne

Overview

- What is function of dystrophin protein
- What happens when there is no dystrophin?
- How can therapeutic approaches prevent/delay this process?
  - Genetic approaches
  - Drugs

Genes and proteins

- Proteins building blocks of our body
- Genes contain blueprint for proteins
- Mistake in gene → mistake in protein
- Genes have a volume switch

  Only on in necessary tissue
- Dystrophin protein has function in muscle
- Mistake in dystrophin → muscle problems

Muscles

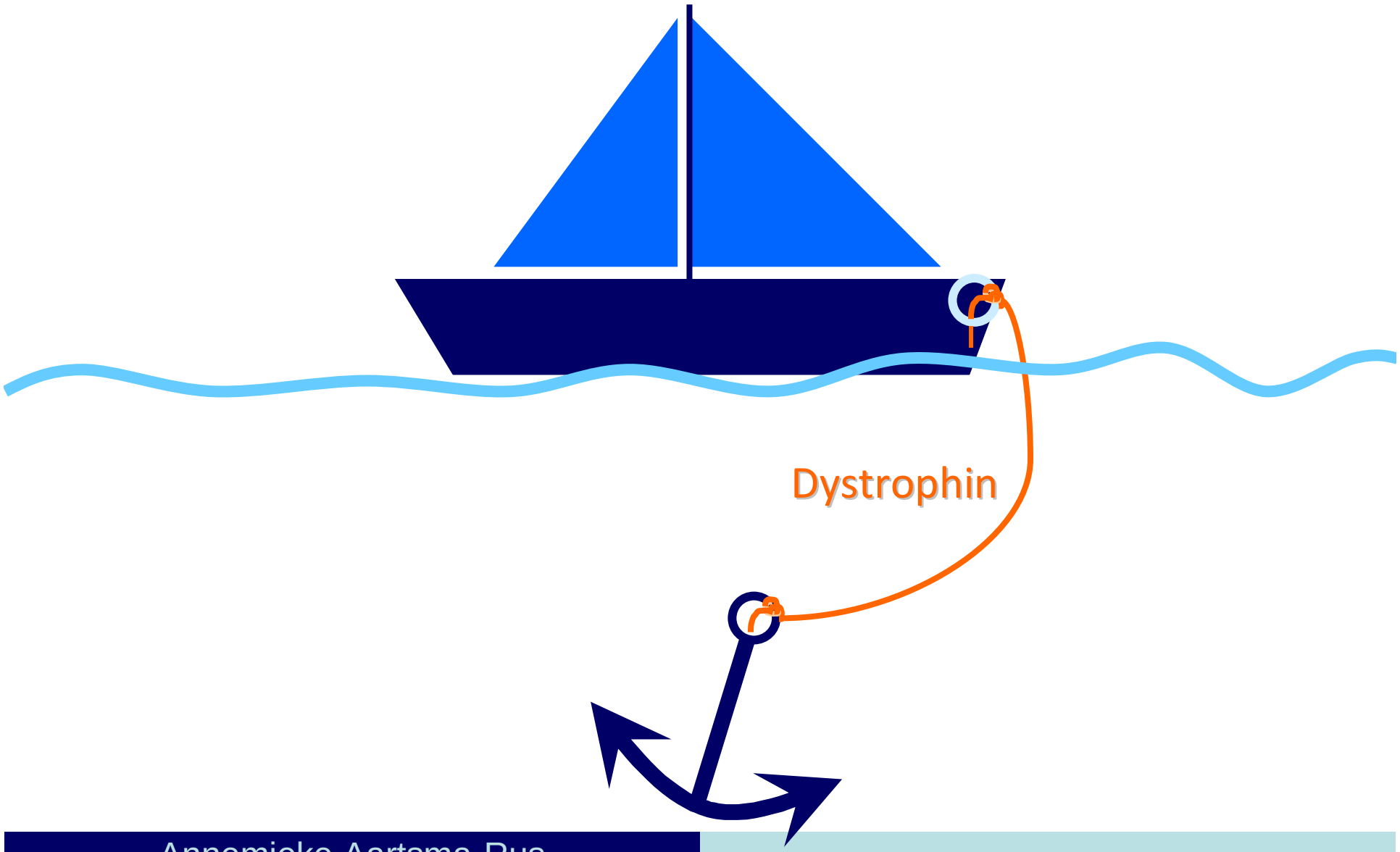
- 30-40% of our body consists of muscle
- Muscles can grow bigger and smaller
- Muscles use lot of energy
- Only maintained when needed
- Muscles damaged after excessive exercise
- Muscles very efficient at repairing damage

➔ Bigger when needed

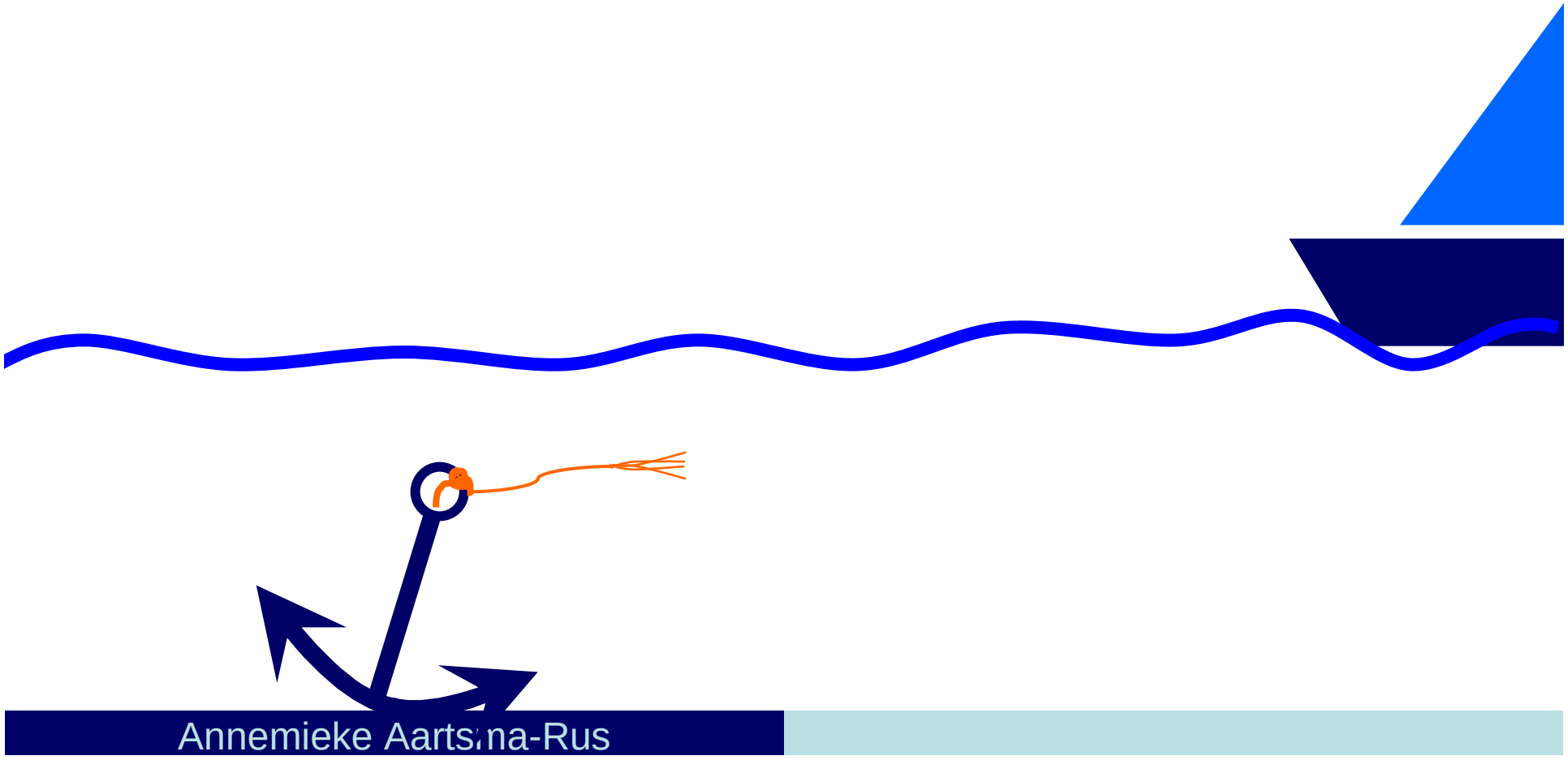
Dystrophin

- Dystrophin provides stability to muscle cells during exercise
- Link between skeleton inside muscle cell and connective tissue outside muscle cell
- Link lost: muscle cells damaged during exercise
- Repair system cannot keep up
- Loss of muscle mass and muscle function

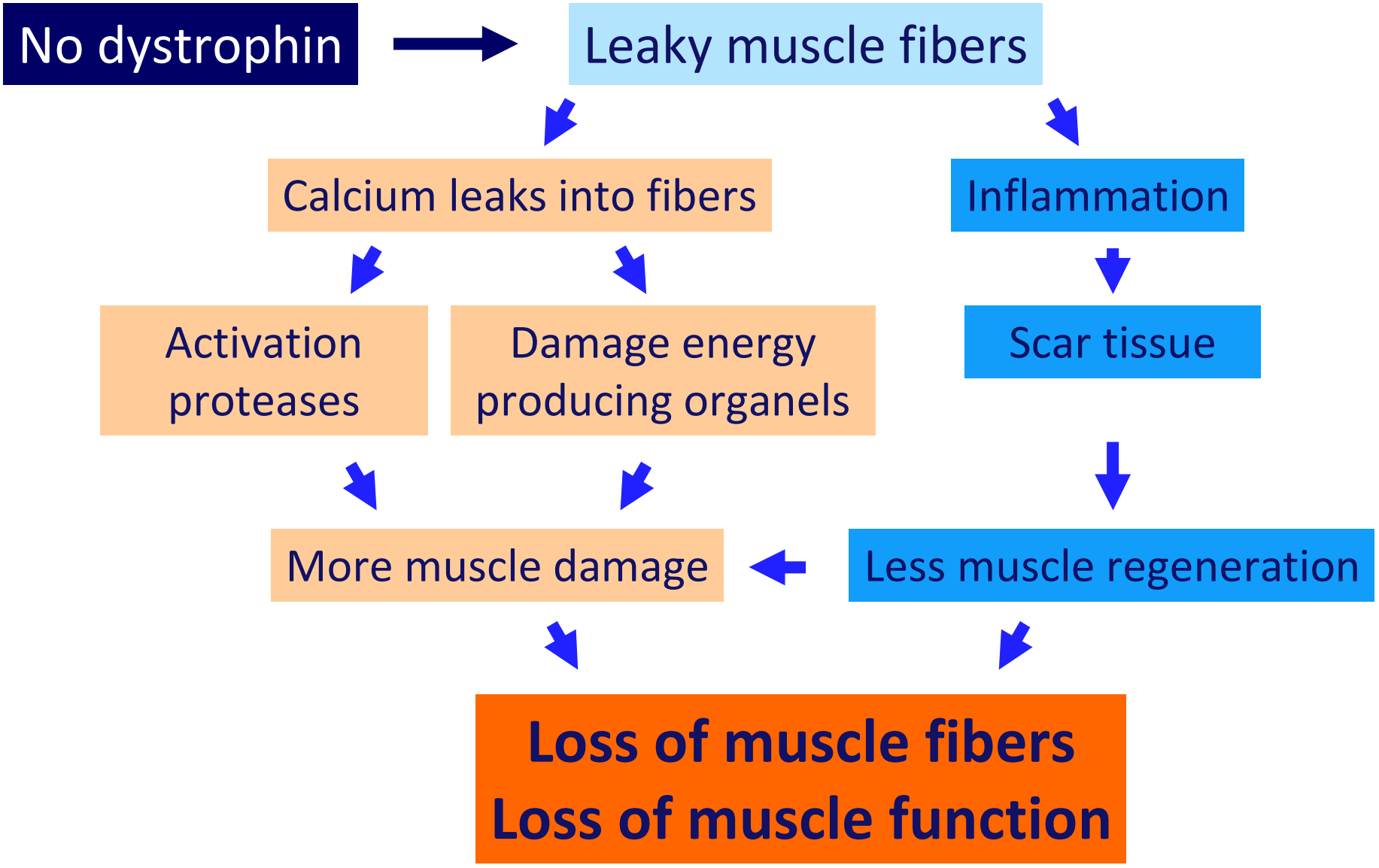
Dystrophin



Duchenne: no functional dystrophin



No functional dystrophin



Therapeutic approaches

- Gene therapy
- Cell therapy
- Genetic therapy
  - Exon skipping
  - Stop codon readthrough
- Drug therapy
  - Utrophin upregulation
  - Myostatin inhibition

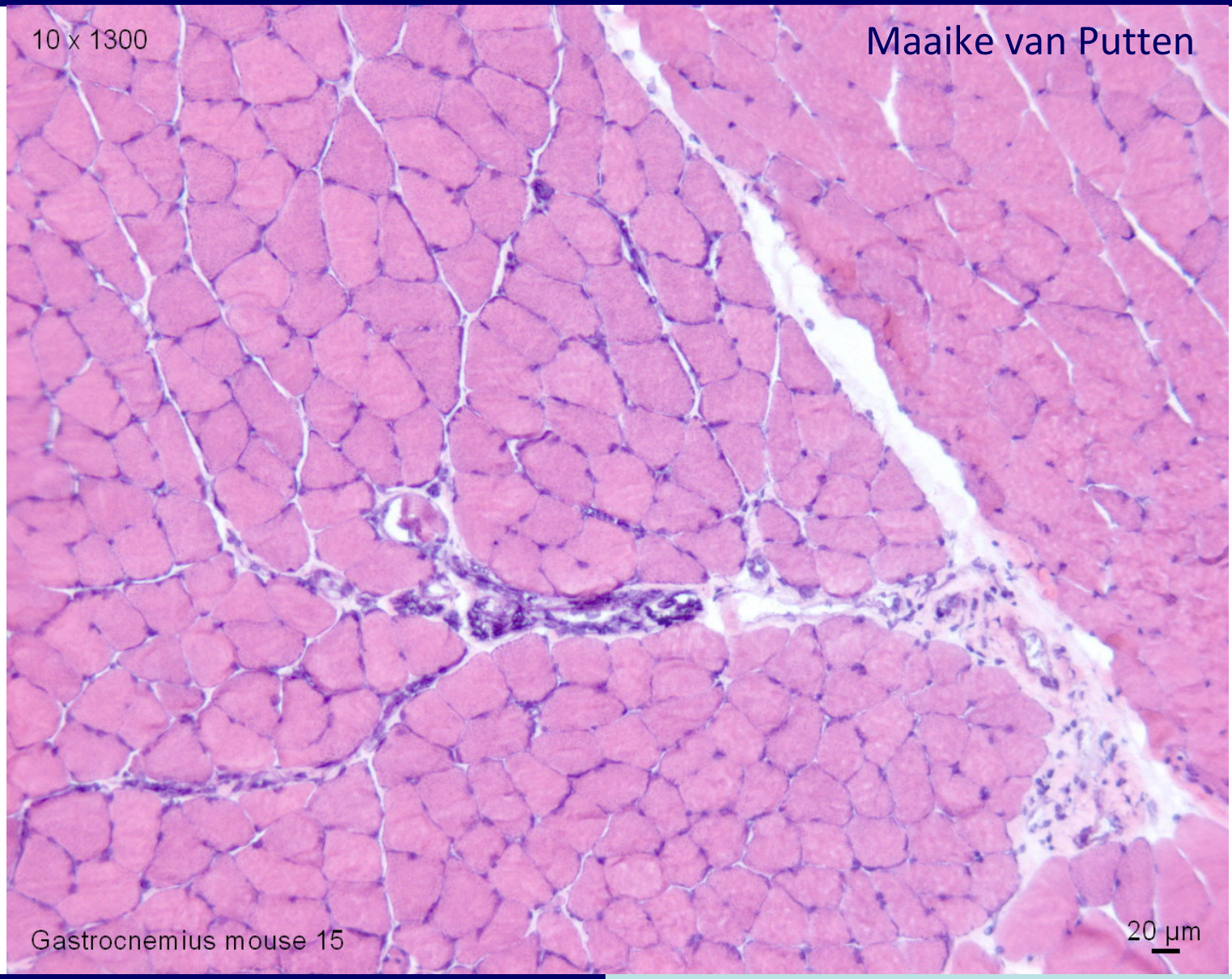
Restore/compensate for dystrophin

More muscle mass

Gene Therapy

- Add functional gene to muscle cells patients
- Dystrophin protein made from new gene
- Applicable to ALL patients
- Genes located in nucleus cells
- How to get gene into (majority) nuclei of muscle cells?

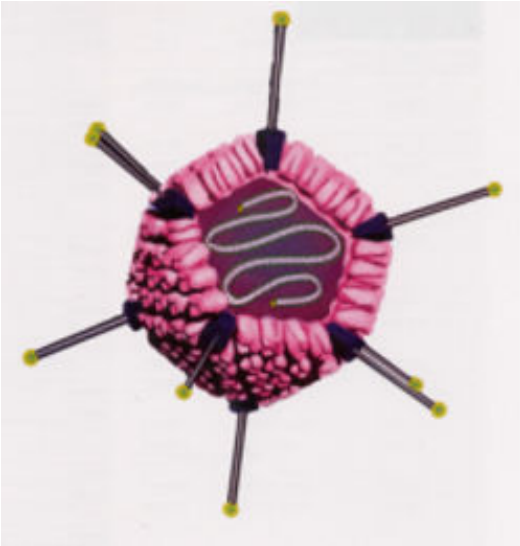
Gene Therapy



Gene Therapy

Virus

- Small organism that injects genetic information into cells
- Use to deliver dystrophin gene
- Adapt
  - Remove virus genes (pathogenic)
  - Add new gene (dystrophin)



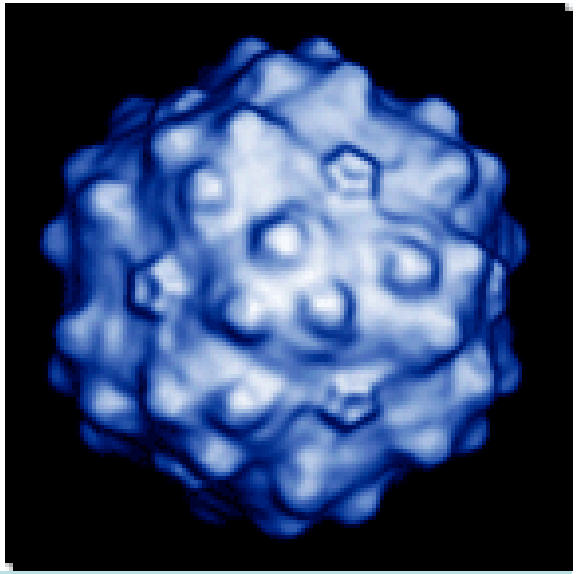
Gene Therapy

Which virus?

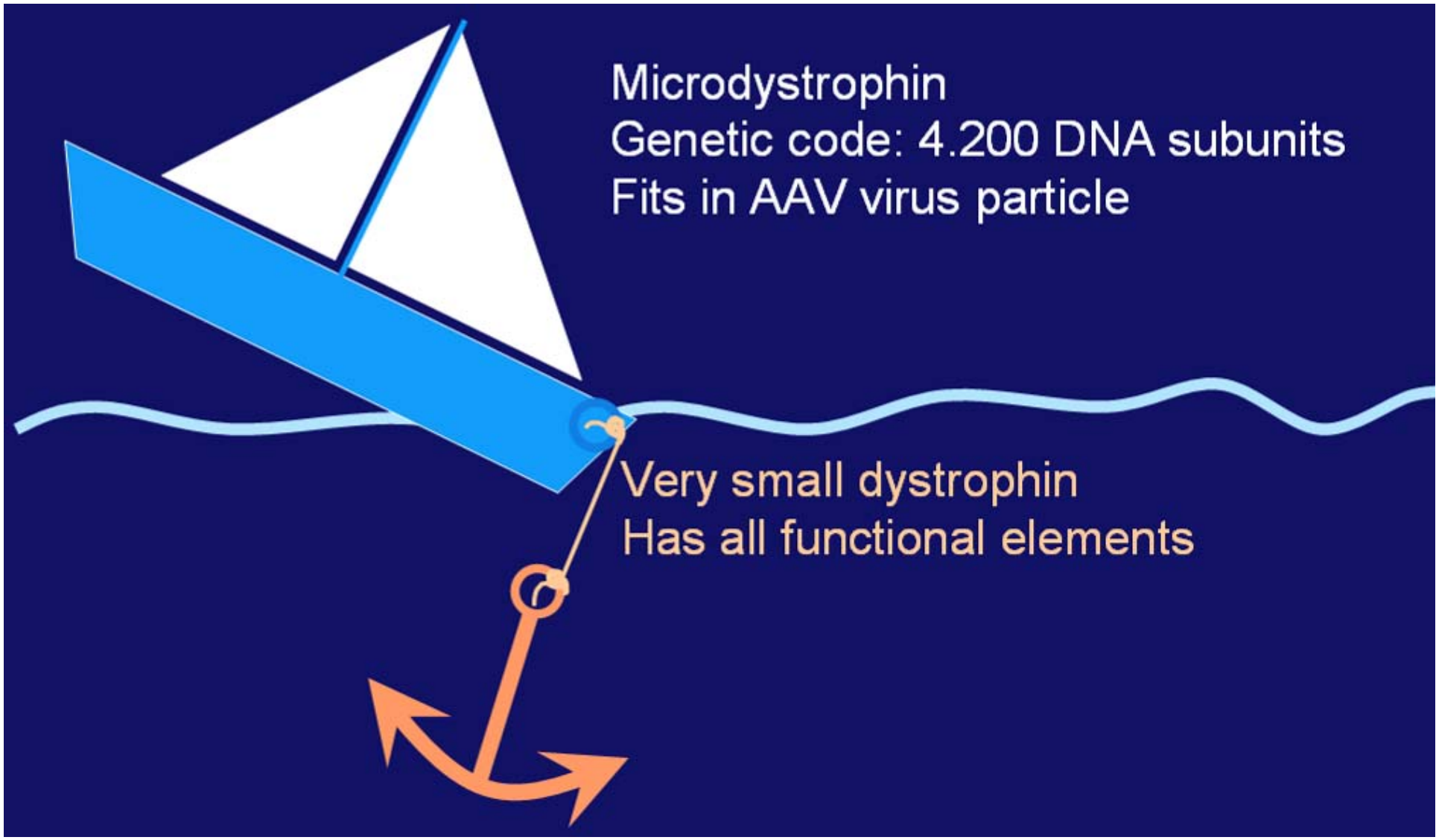
- Most viruses do not infect muscle tissue
  - Muscle cells do not divide often
  - Lot of connective tissue (filters out viruses)
- Exception: adeno-associated virus (AAV)
- Preference for muscle
- Not pathogenic in man

Gene Therapy

- Very small (20 nm, 0.00002 mm)
- Capacity: 4.500 DNA subunits
- Dystrophin gene: 2.200.000 DNA subunits
- Genetic code gene: 14.000 subunits
- Remove part from genetic code
- Only essential parts remain

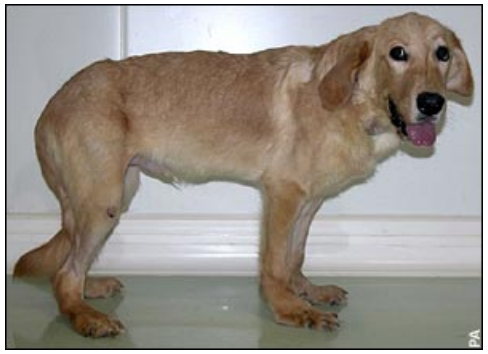


Gene Therapy



Gene Therapy

- AAV microdystrophin tested in mdx mouse model
- Microdystrophin detected in muscle!
- Improved muscle function and quality!
- Tested in Duchenne dog model



- Immune problems (virus)
- AAV also induces immune problems in humans

Gene Therapy

Clinical trials

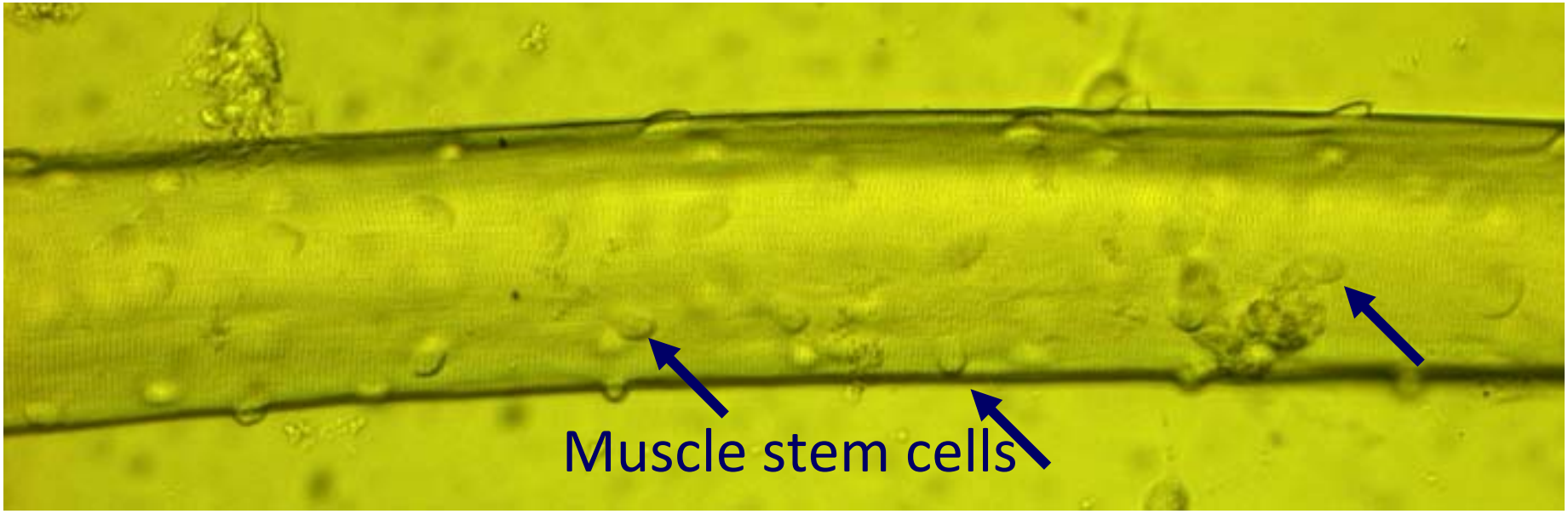
- Safety study in Duchenne patients
- 2006/7, USA: local injection biceps (Mendell,Samulski, Xiao Xiao)
- Immune response!
- Dystrophin in 2/6 patients (low levels)
- Prepare for bigger trial (whole muscle treatment)
- Chamberlain also preparing trial

Gene Therapy

Immune problem

- Other AAV subtypes may not be recognized by immune system
- Immune suppression
  - Only before and immediately after treatment?
- Use only DNA (Jon Wolff, France)

Cell therapy

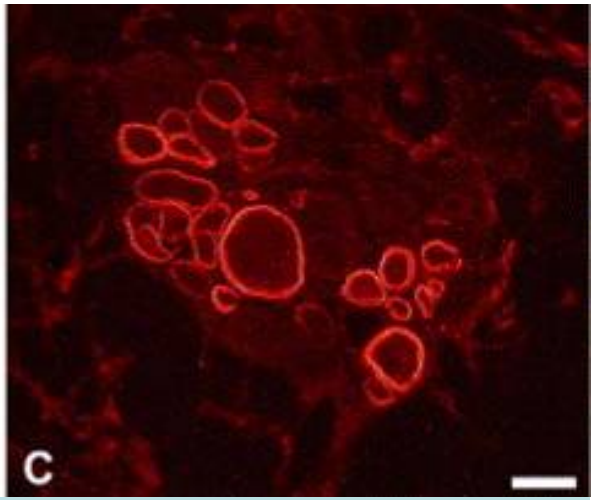


- Isolate muscle stem cells from healthy donor
- Expand outside the body (culture in lab)
- Transplant into patients

Cell therapy

Problem

- Immune response (suppress)
- Muscle stem cells do not migrate from bloodstream into muscle
- Muscle stem cells do not migrate IN muscle (stay close to injection site)
- Multiple injections (Tremblay, Canada)



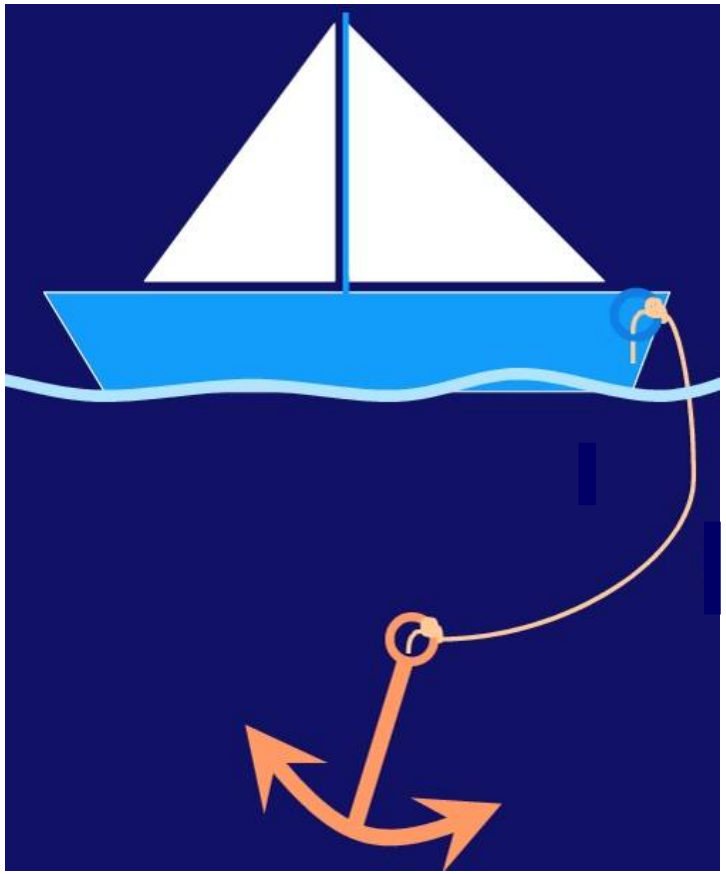
Cell therapy

Other stem cells

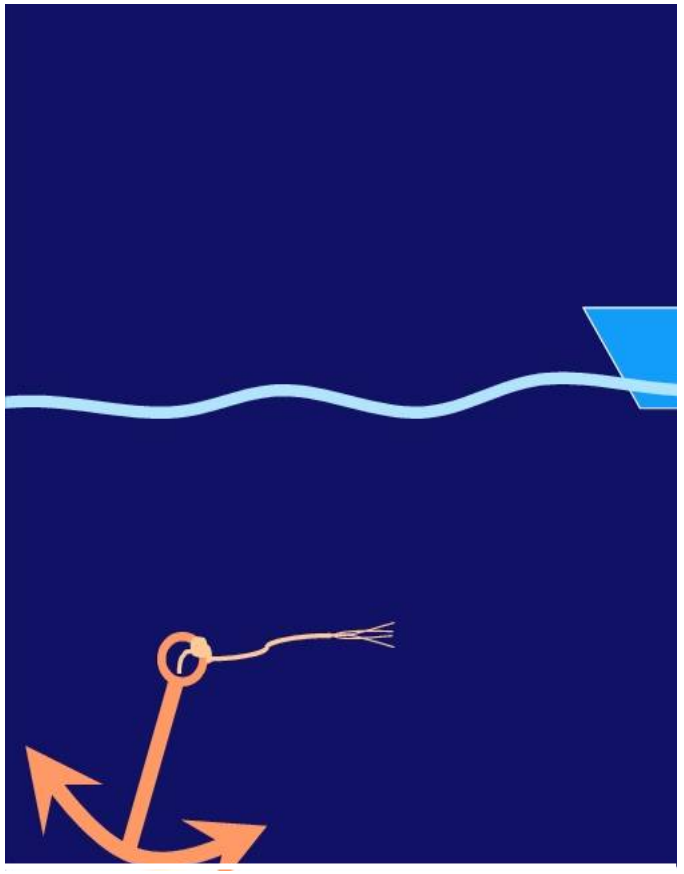
- E.g. blood, blood vessel and adipse (fat) stem cells can become muscle cells
- Can migrate from blood into muscle
- Efficiency currently very low
- Mesangioblasts and CD133+ cells promising (more efficient)
- Trials planned for early 2011 (Italy)
- Autologous stem cells also studied (no immunity)

Exon skipping

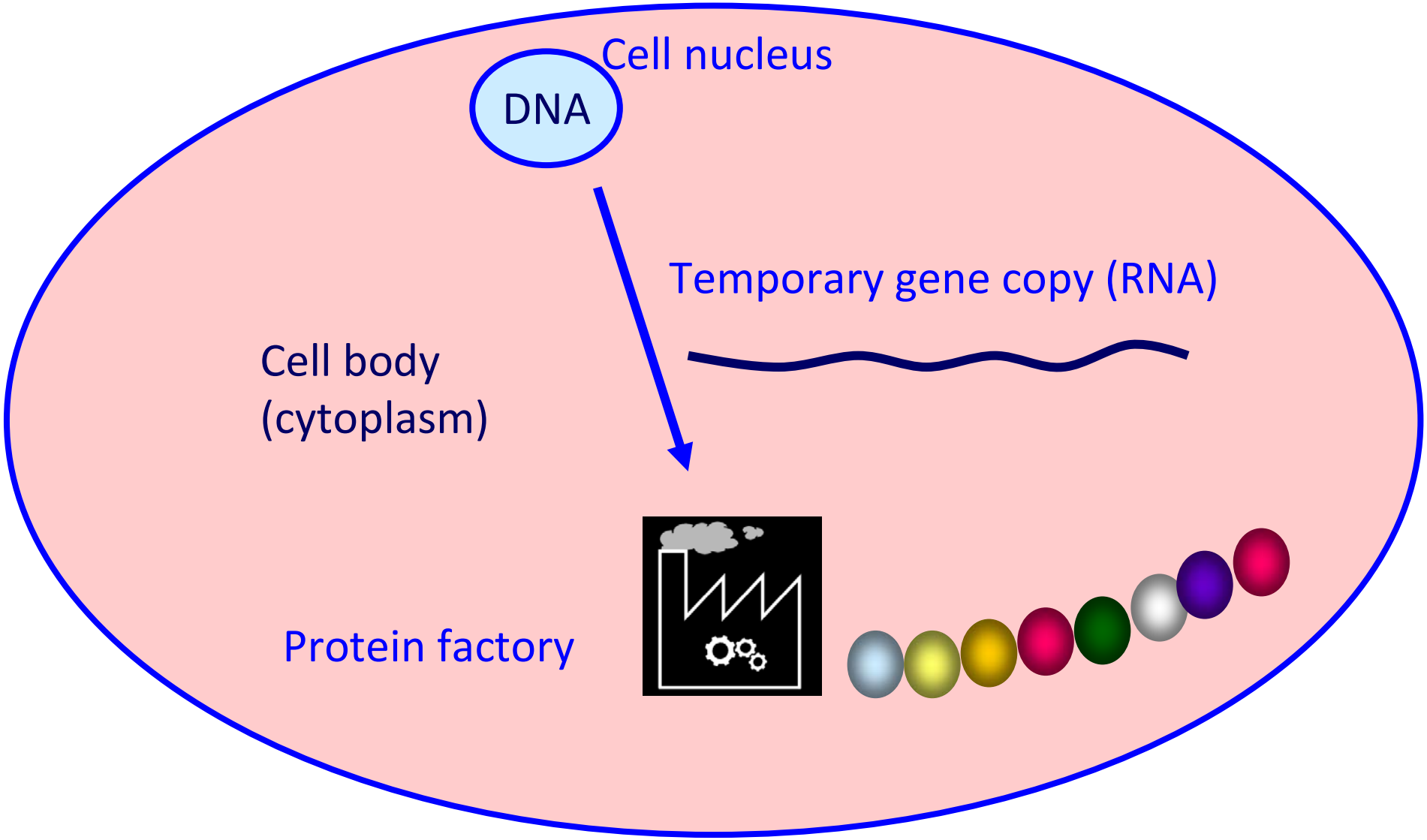
Normal



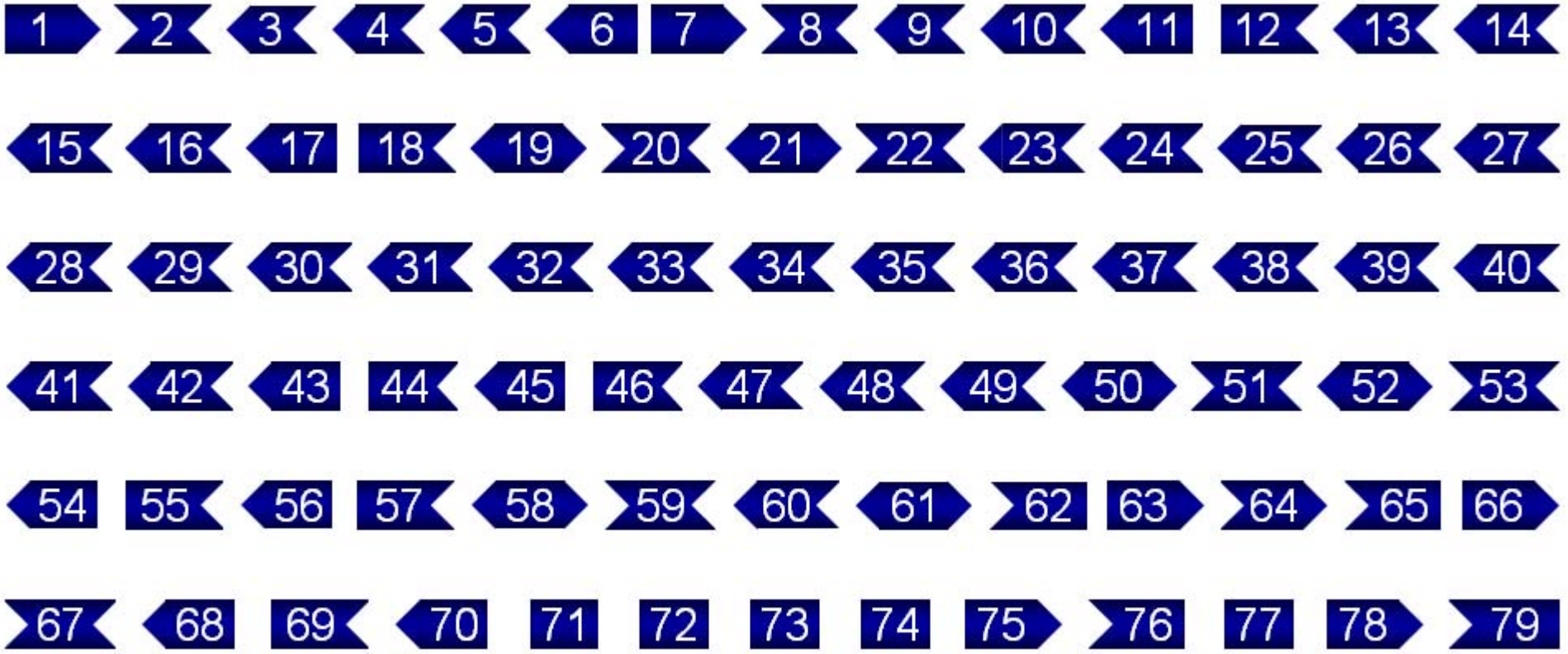
Duchenne



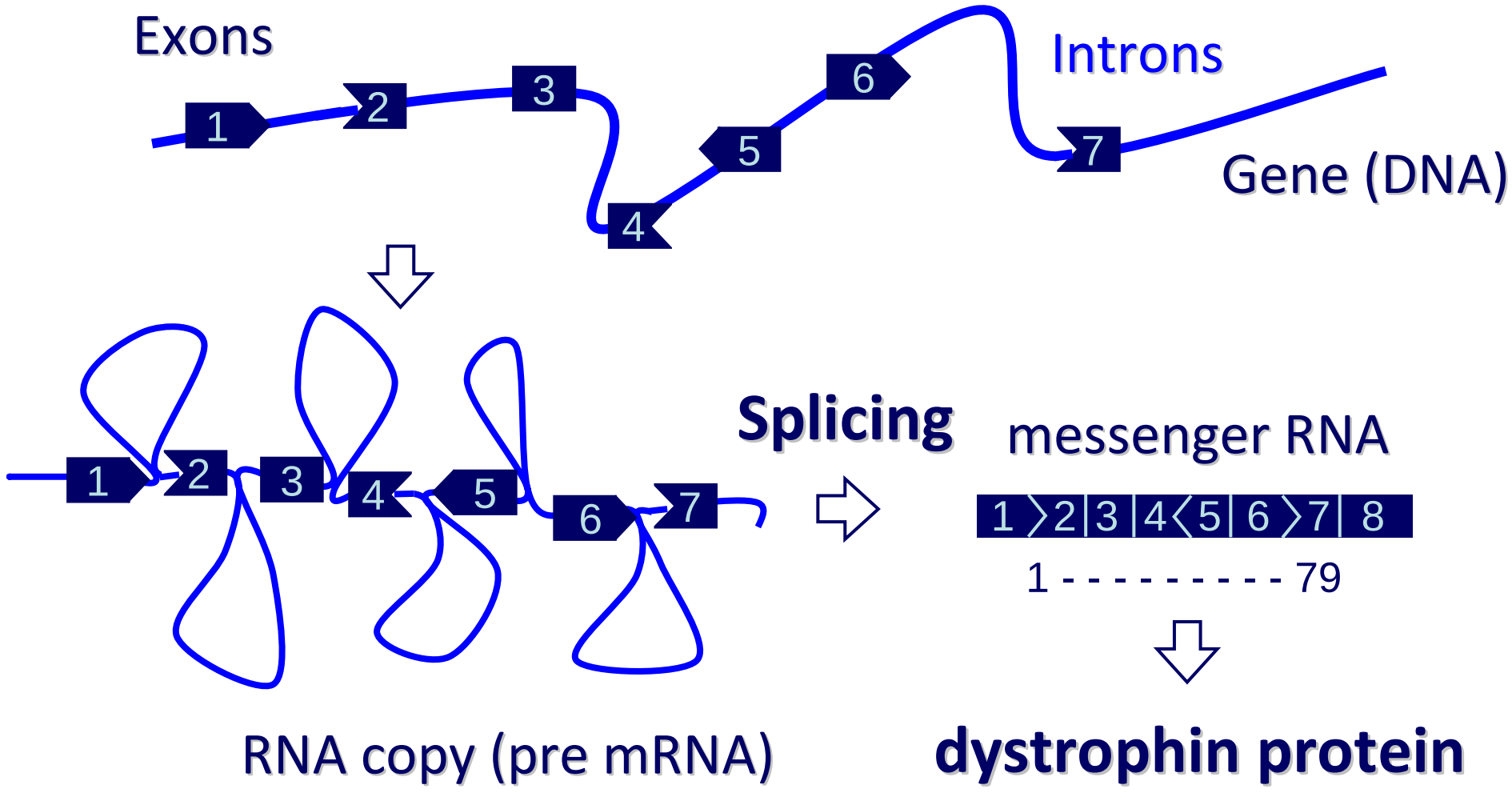
Gene ➔ Protein



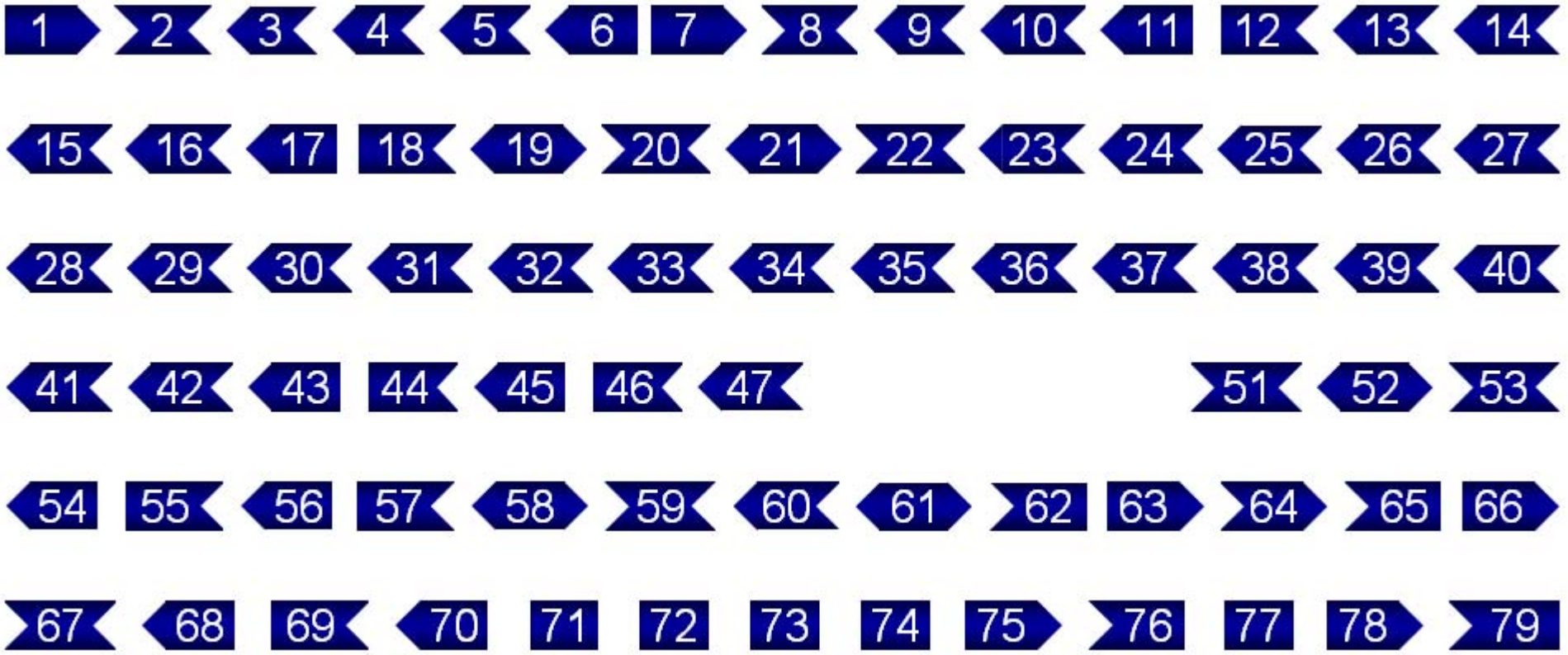
Dystrophin gene



Splicing

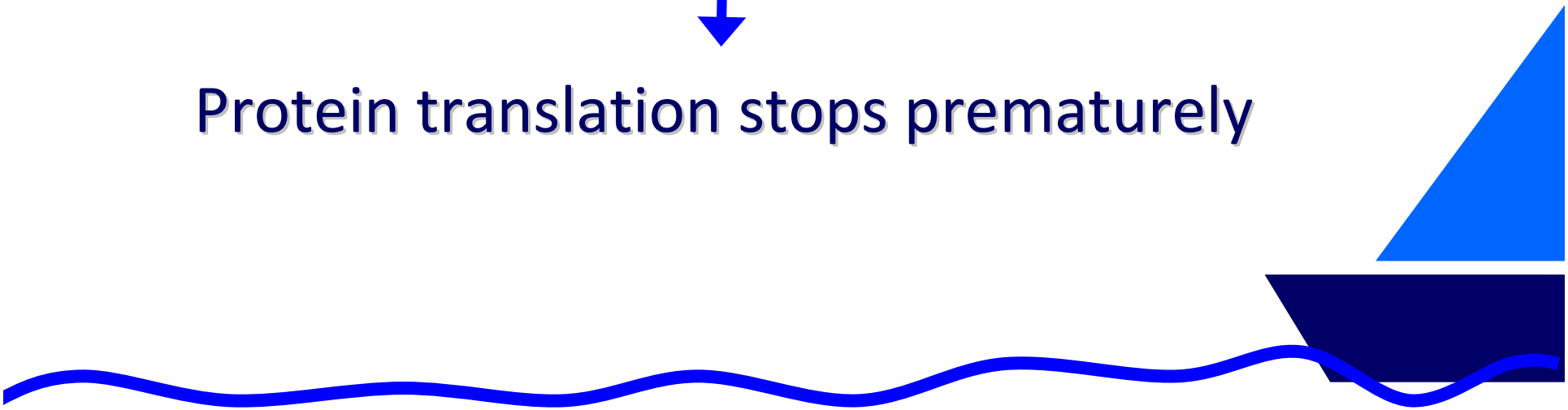

Exons
1
2
3
4
5
6
7
Introns
Gene (DNA)
Splicing
messenger RNA
1 2 3 4 5 6 7 8
1 - - - - - - - - - 79
RNA copy (pre mRNA)
dystrophin protein

Duchenne: genetic code disrupted



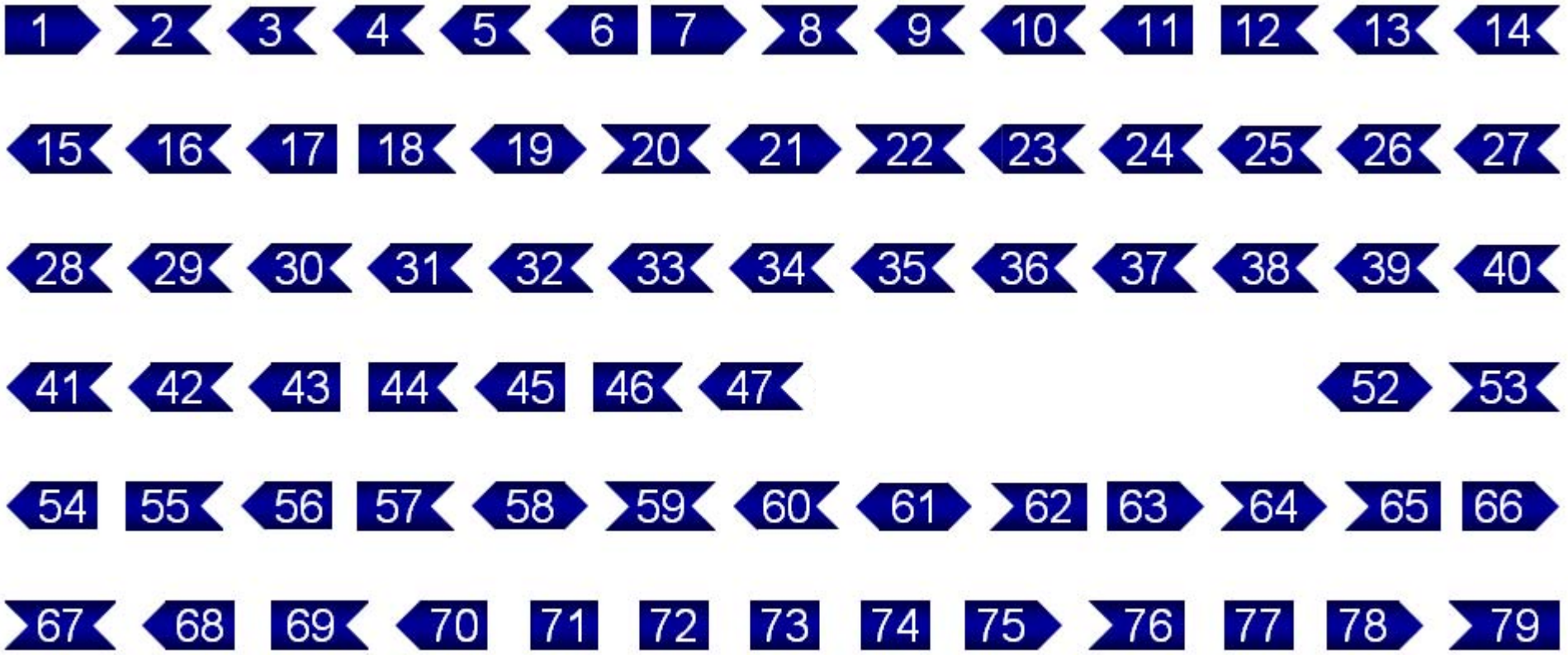
Duchenne: genetic code disrupted

Protein translation stops prematurely

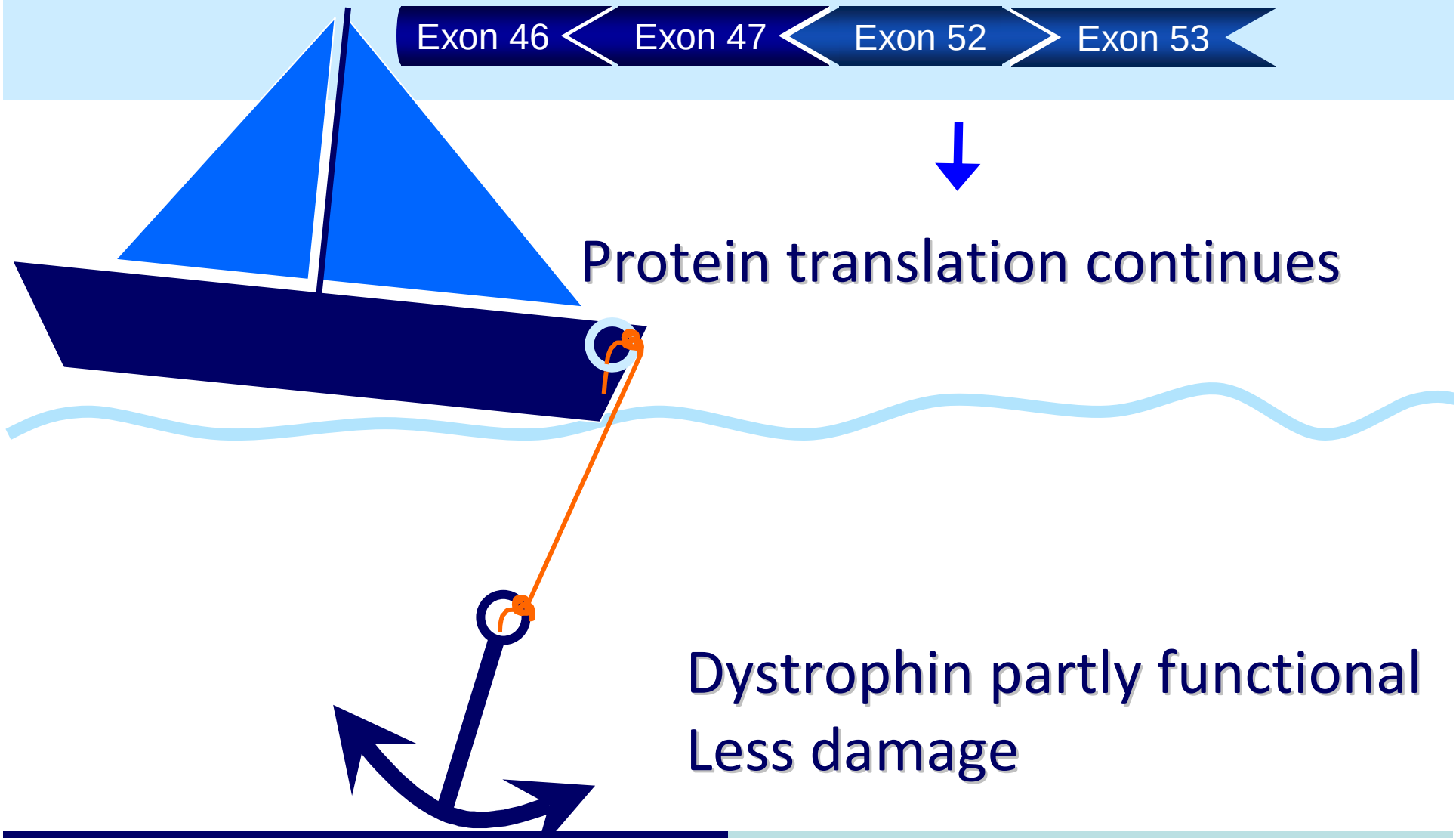


Dystrophin not functional

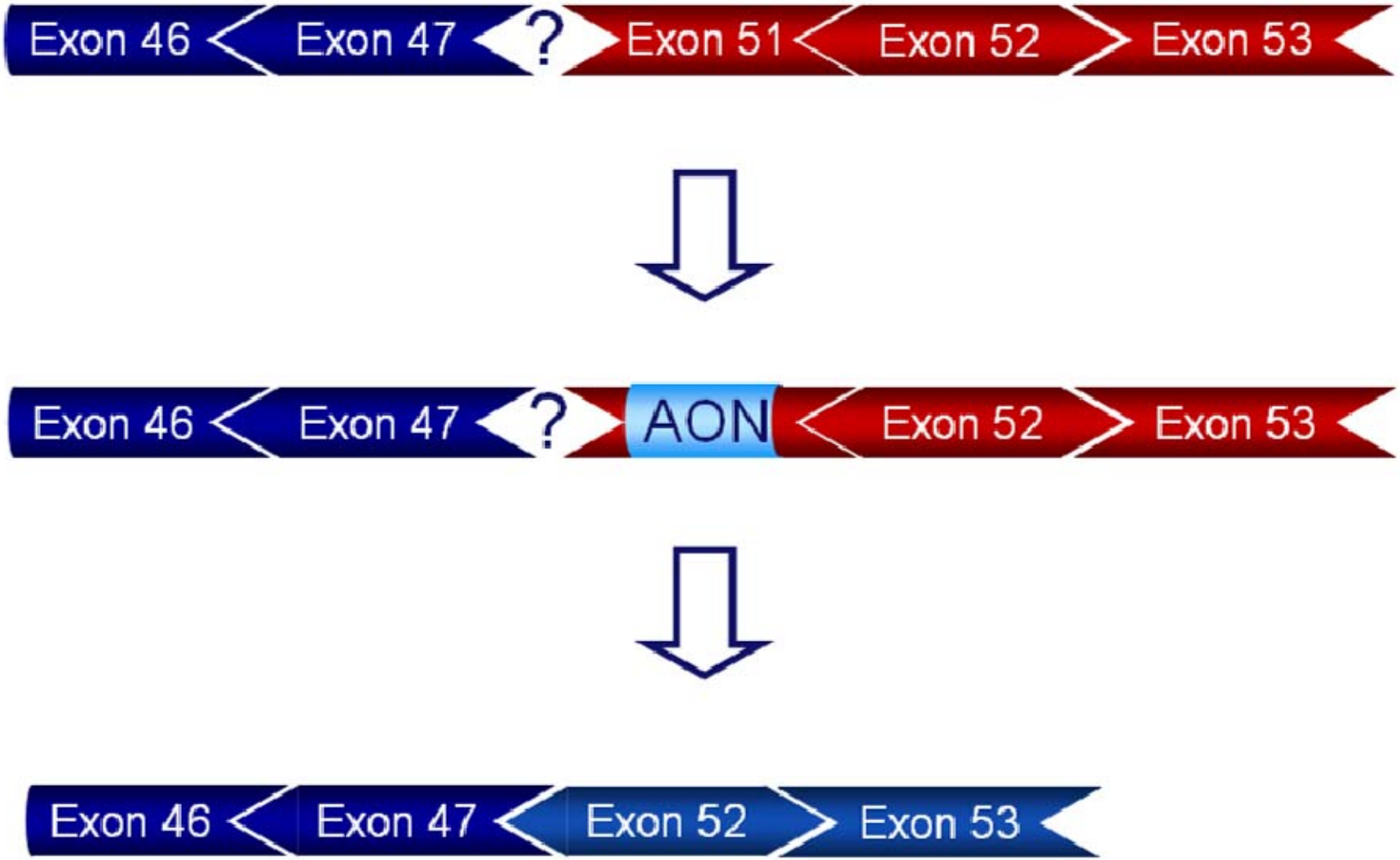
Becker: genetic code maintained



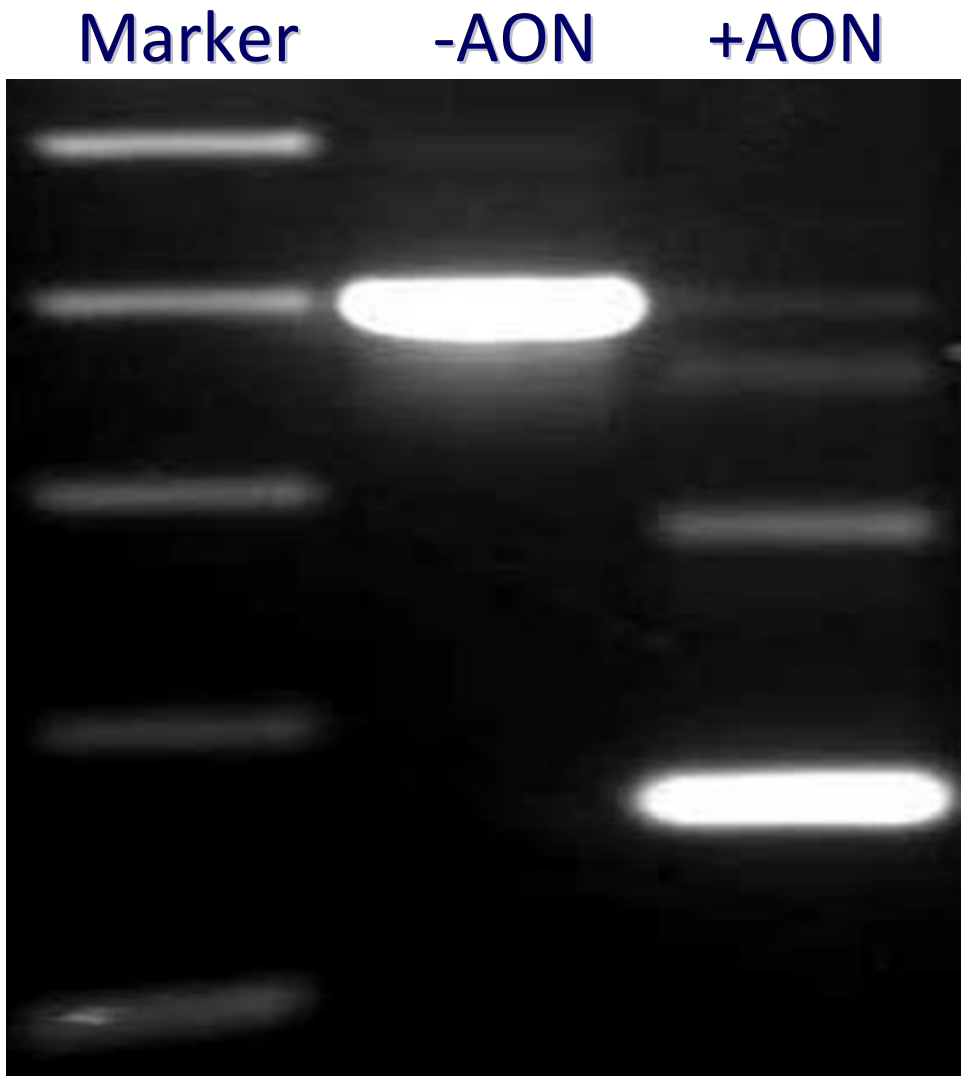
Becker: genetic code maintained



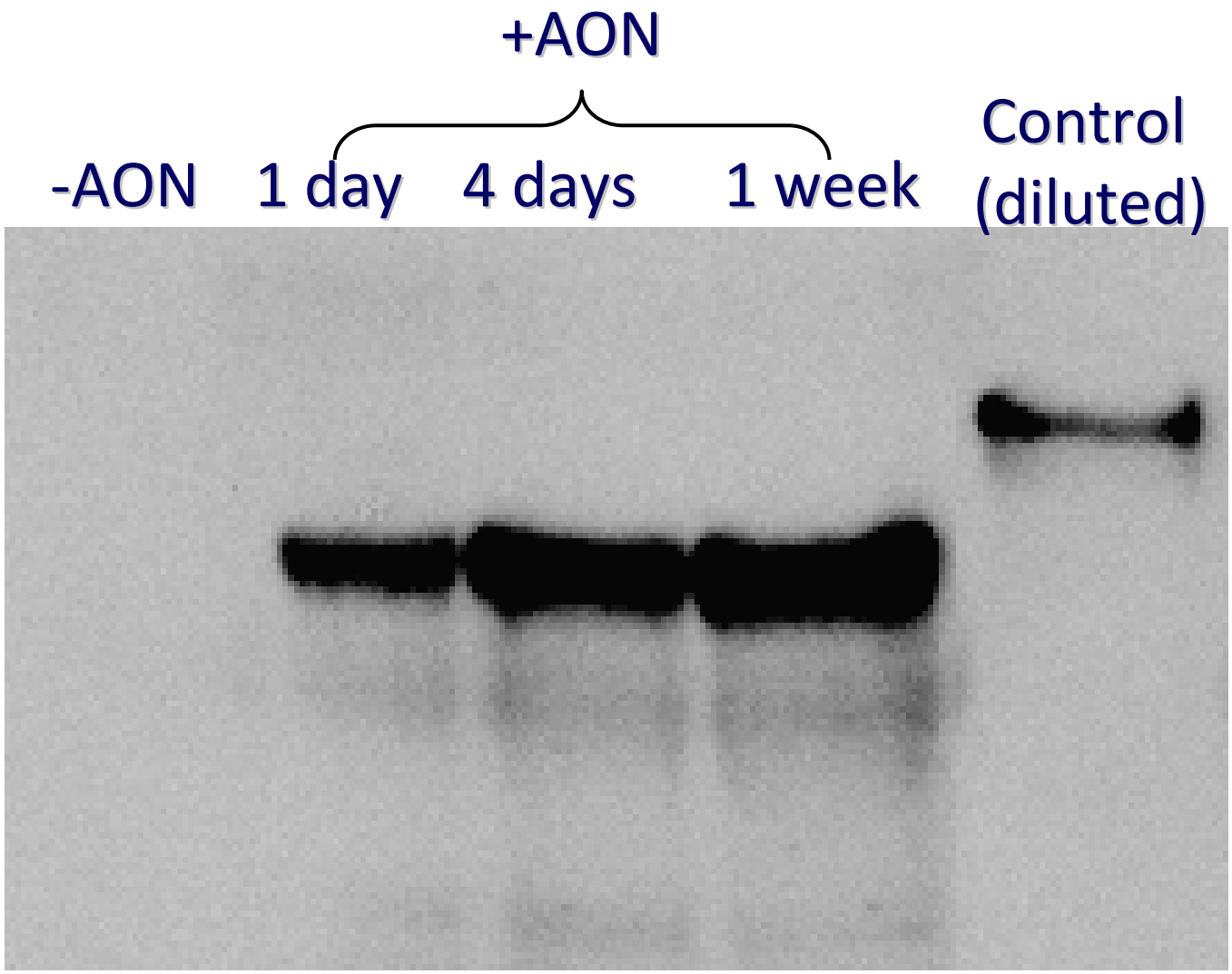
Exon skipping: restore genetic code



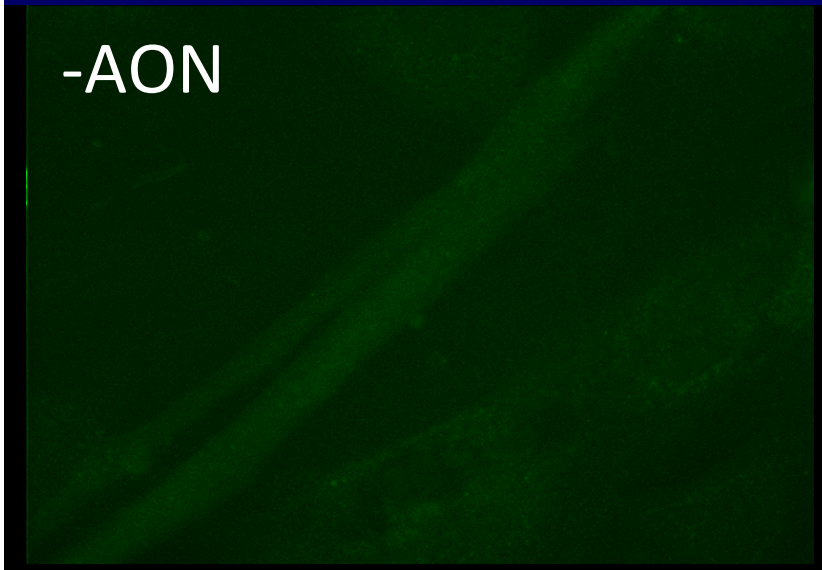
Exon skipping

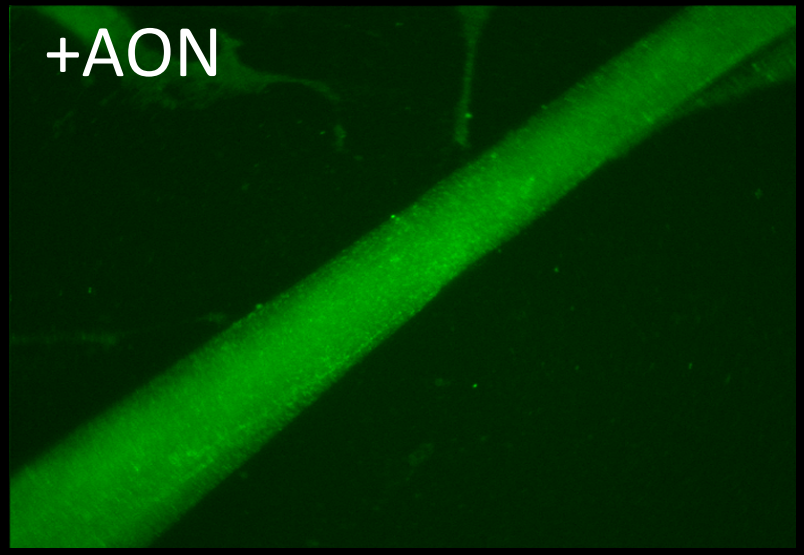


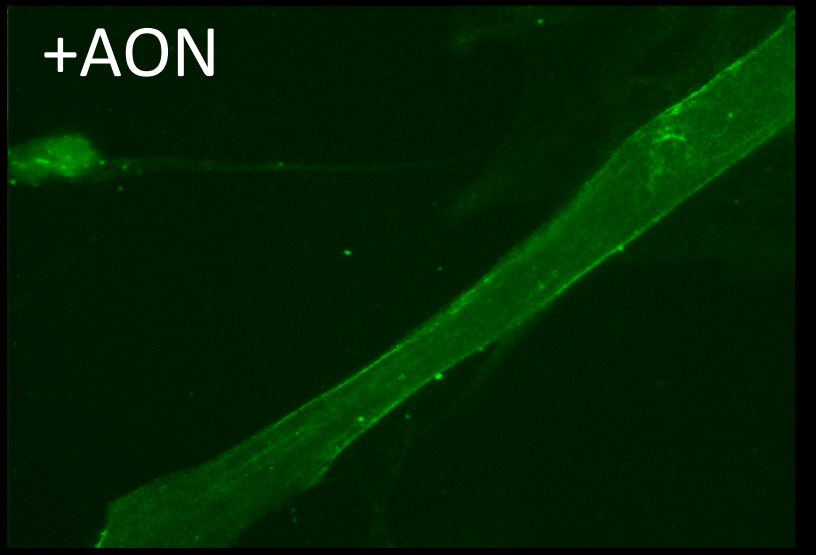
Exon skipping



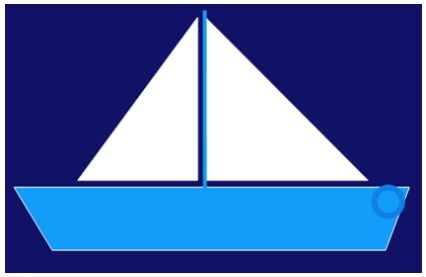
Exon skipping







Applicability

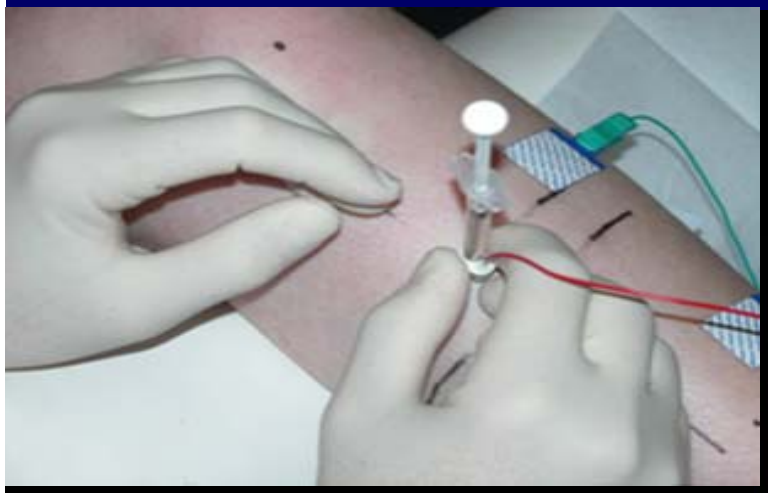


hotspot

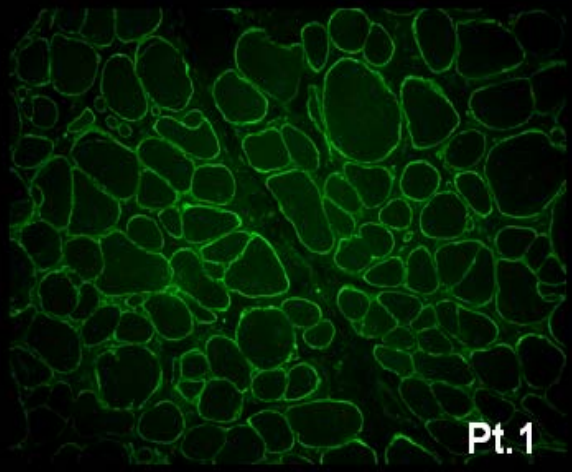
Exon	All mutations	Deletions	Duplications	Small mutations
51	13.0%	19.1%	0.3%	3.0%
45	8.1%	11.8%	0.2%	2.2%
53	7.7%	11.4%	0.1%	1.5%
44	6.2%	8.85	0.4%	2.7%
46	4.3%	6.2%	0.2%	1.6%
52	4.1%	5.7%	0.5%	2.3%
50	4.0%	5.6%	0.2%	1.9%

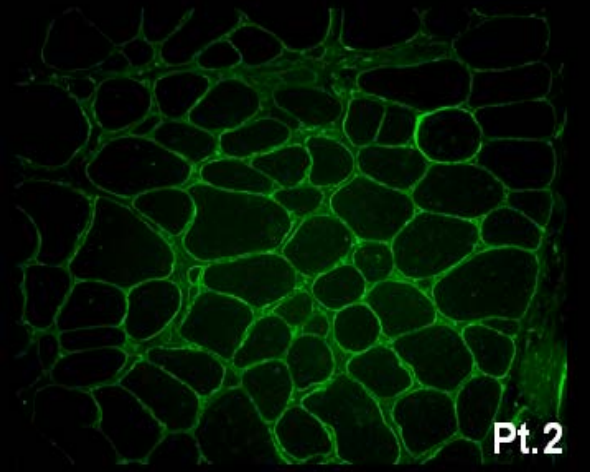
Aartsma-Rus Hum Mutat 2009, 30:293-9

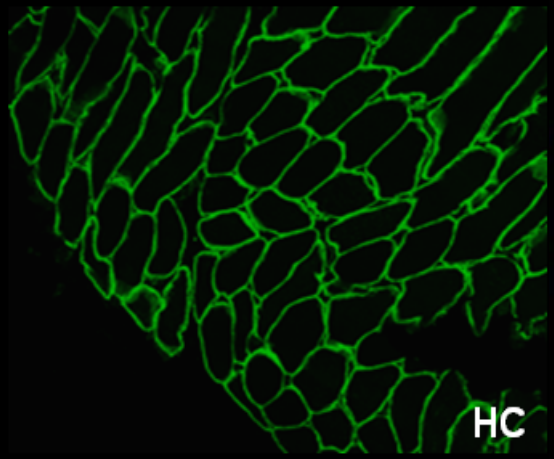
Intramuscular trial (Prosensa)

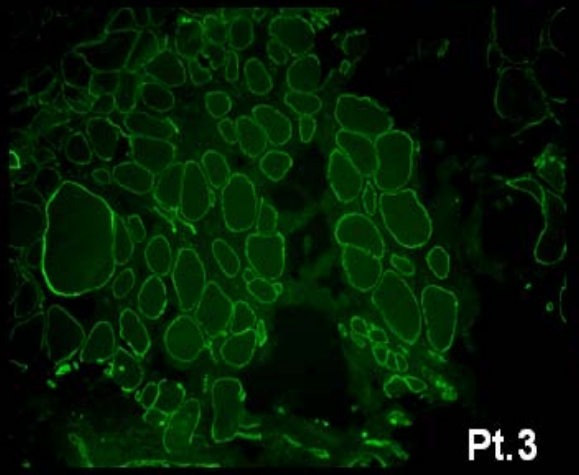


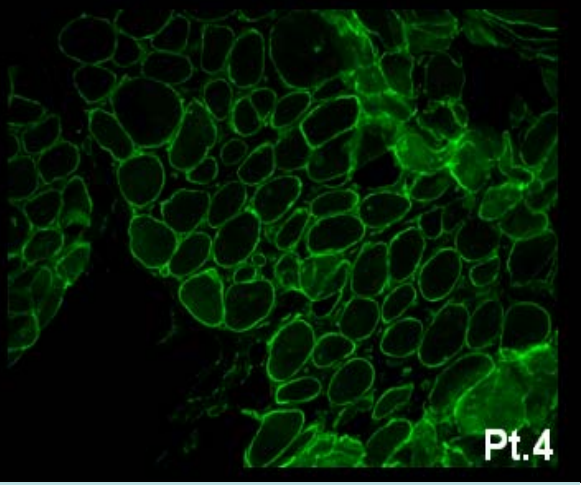
0.8 mg in TA







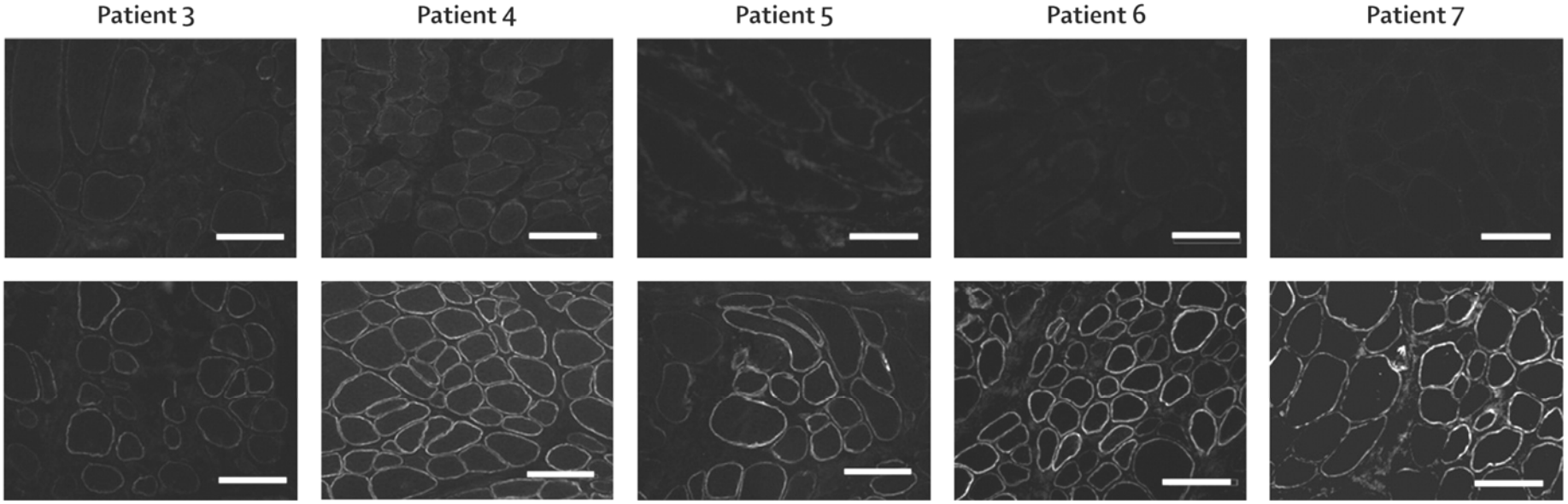




Van Deutekom NEJM 2007; 357: 2677-86

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

Intramuscular trials

- Exon skipping observed in all patients
- No toxic effects observed!
- Dystrophin levels very comparable

  17-35% vs 22-32%
- Number of dystrophin positive cells comparable

  64-97% vs 44-79%
- Systemic treatment needed

Systemic trials

Prosensa trial (Belgium/Sweden)

- 12 patients, 4 dose groups (0.5, 2, 4 & 6 mg/kg)
- Subcutaneous weekly injections for 4 weeks
- 6 month extension trial ongoing (6 mg/kg/week)

AVI trial

- 19 patients, 6 dose groups (0.5, 1, 2, 4, 10 & 20 mg/kg)
- Intravenous weekly injections for 12 weeks
- Extension trial planned

Systemic trial results

Prosensa trial

- Dystrophin restoration in a dose dependent way
- Homogeneous staining
- No toxic effects observed
- Extension trial ongoing

Systemic trial results

AVI trial

- Dystrophin restoration
- Response differs within dose groups
- Three patients respond very well (2, 10 & 20 mg/kg)

  Up to 50% dystrophin positive fibers highest dose!
- No toxicity observed

Planned/ongoing trials

- Exon 51 skipping
  - Non ambulant trial planned (Prosensa)
  - Dose finding trial planned (Prosensa)
  - Phase 3 trials planned (GSK/Prosensa)
- Exon 44 skipping Phase I/II started (Prosensa)
- Exon 45 skipping trial planned (Matsuo, Japan)
- Optimization other exons ongoing
- Dialogue with regulatory agencies initiated

PTC124/ataluren

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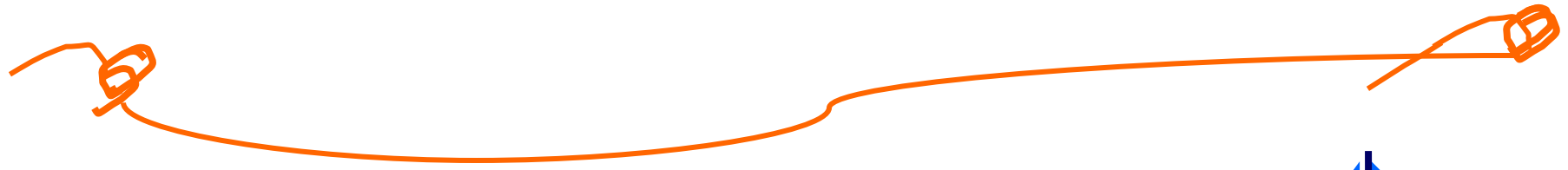
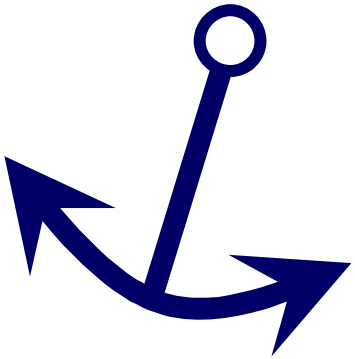
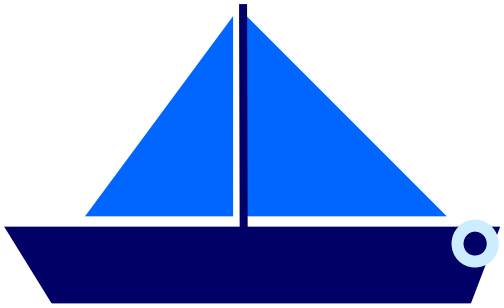
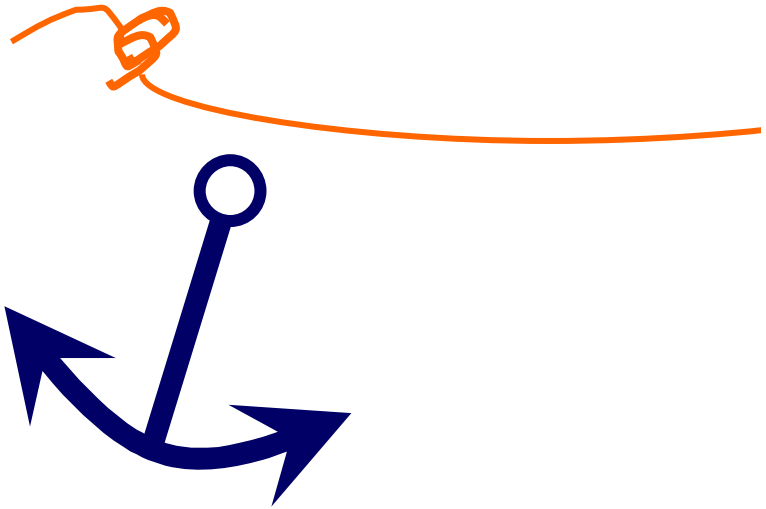


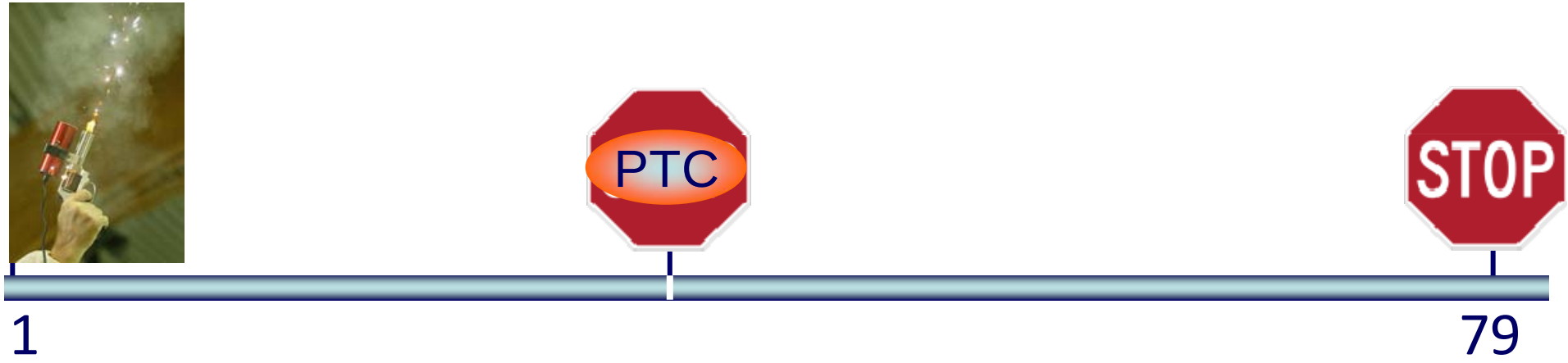




PTC124/ataluren



PTC124/ataluren

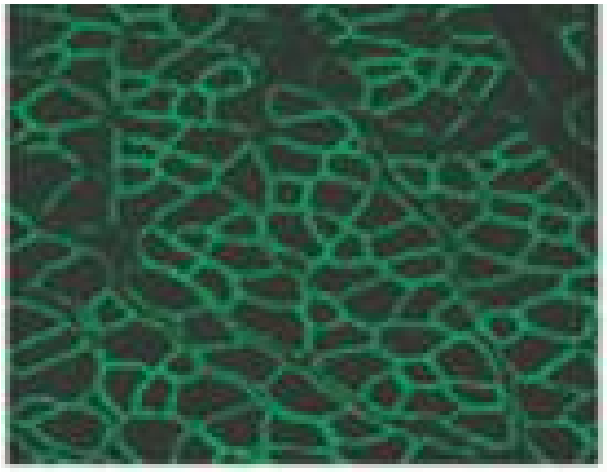


Cell ignores new stop signal
Complete protein is made

PTC124/Ataluren

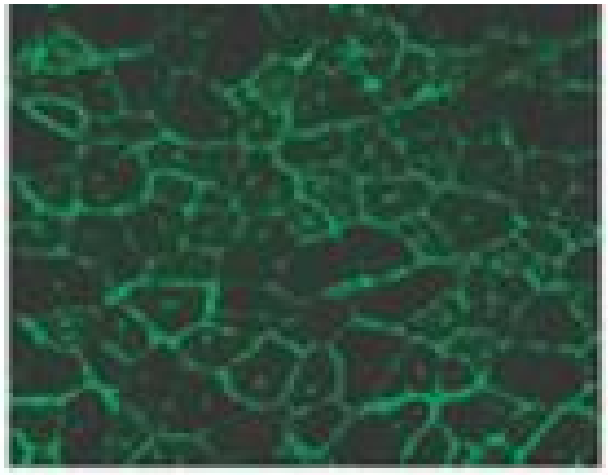
- Tested in patient-derived cells
- Tested in *mdx* mouse model
- Dystrophin restoration

Control



Mdx

Mdx + PTC124



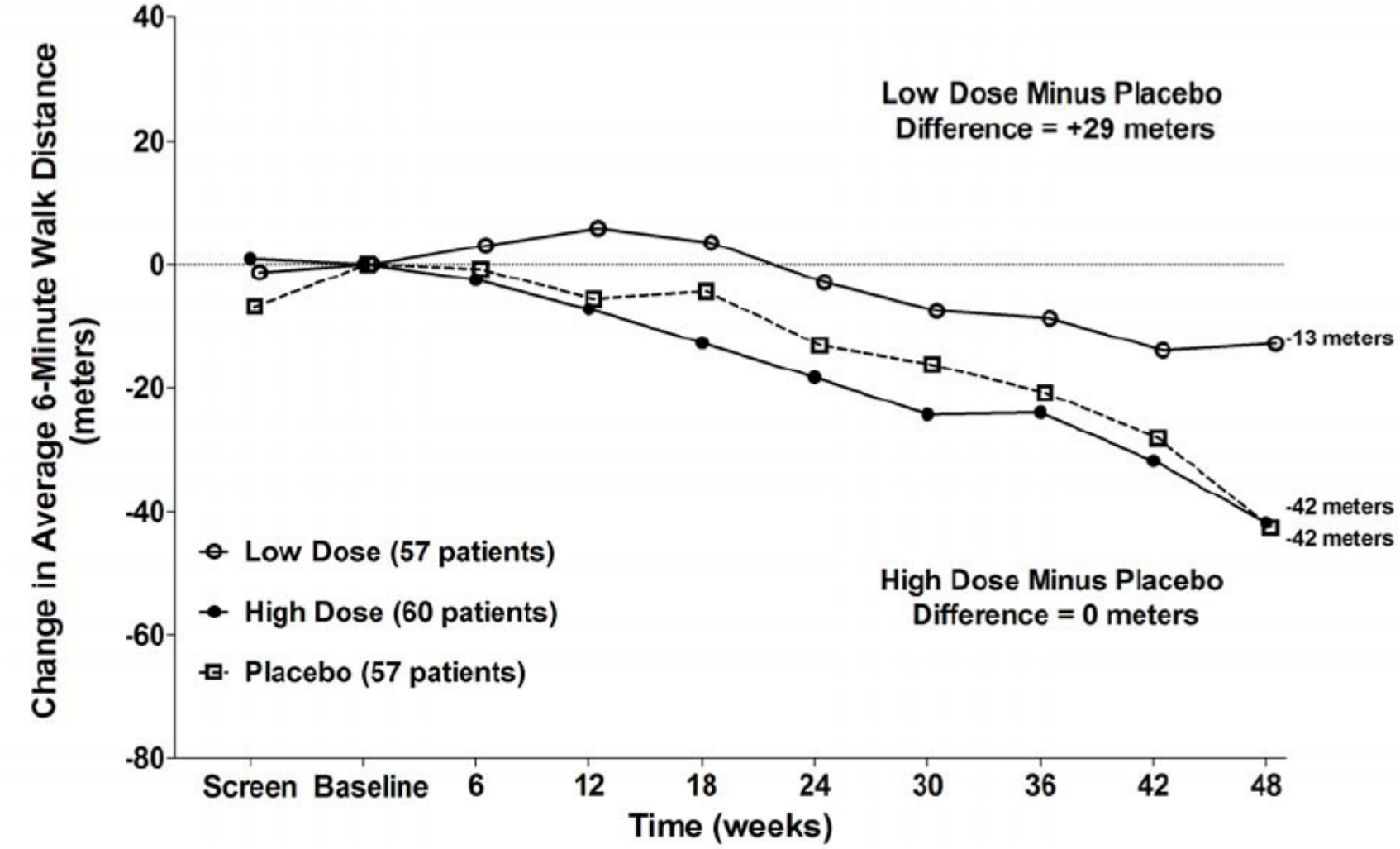
Welch et al, Nature 2007, 447: 87-91

PTC124/Ataluren

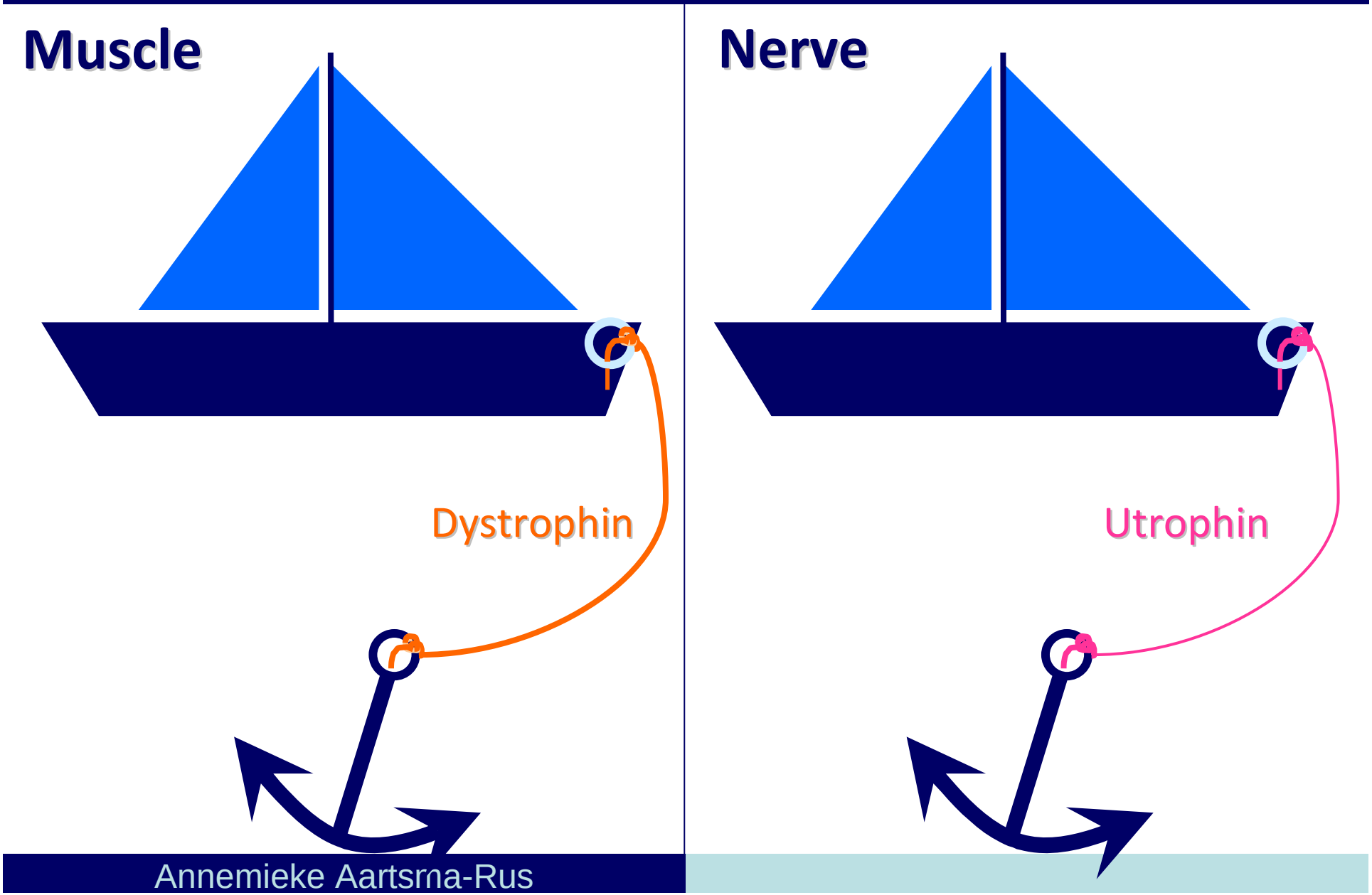
- Tested in healthy controls: safe
- Tested in 28 patients (dose finding)
  - Safe
  - Increased dystrophin expression
- Tested in 174 patients in 48 week trial
  - Placebo, high dose and low dose
  - Safe!
  - No significant difference in primary outcome (6MWT)
  - Dystrophin levels? (analysis pending)

PTC124/Ataluren

Figure 1: Difference between the treatment groups in the average 6MWD through 48 weeks of treatment



Utrophin upregulation



Utrophin upregulation

- Utrophin resembles dystrophin
- Utrophin can take over dystrophin function
- Expressed in nerve cells, hardly in muscle
- Find ways to turn on utrophin gene in muscle cells

Screen drugs to find one that “fits” on the volume switch and switches it on

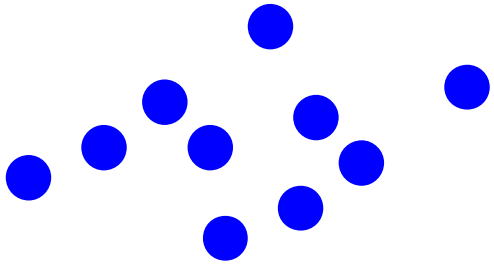
Utrophin gene volume switch

Utrophin upregulation

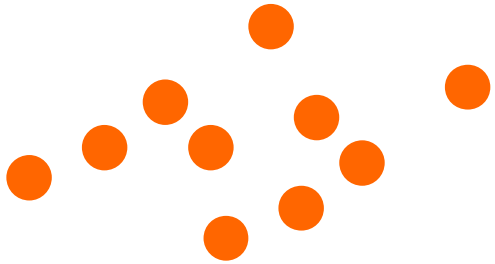
- Screen for drugs that can turn up utrophin volume switch
- High throughput screening in cell models (thousands!)
- Potential drugs screened further in patient-derived cell cultures and mouse models
- Candidate drug currently tested in healthy volunteers (BMN195) by Biomarine

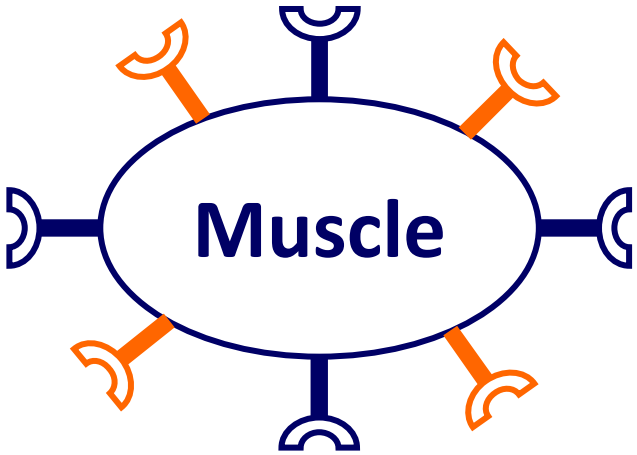
Myostatin inhibition

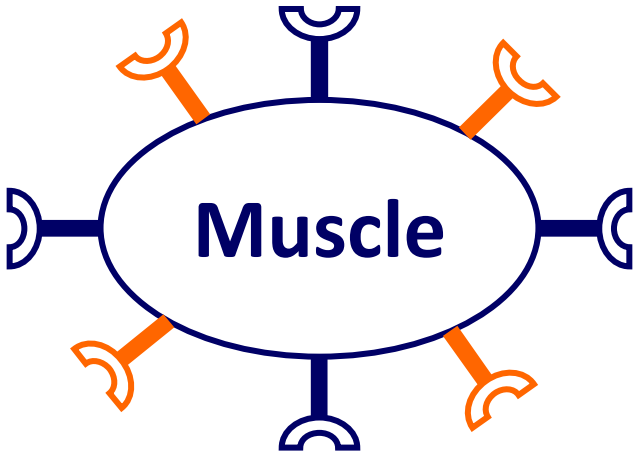
Muscle growth factors



Muscle growth inhibitors

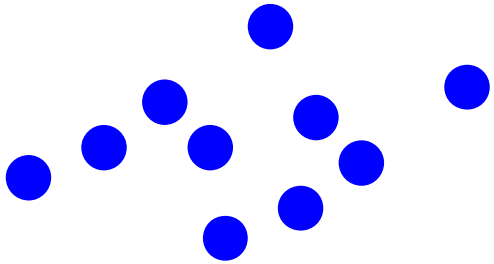


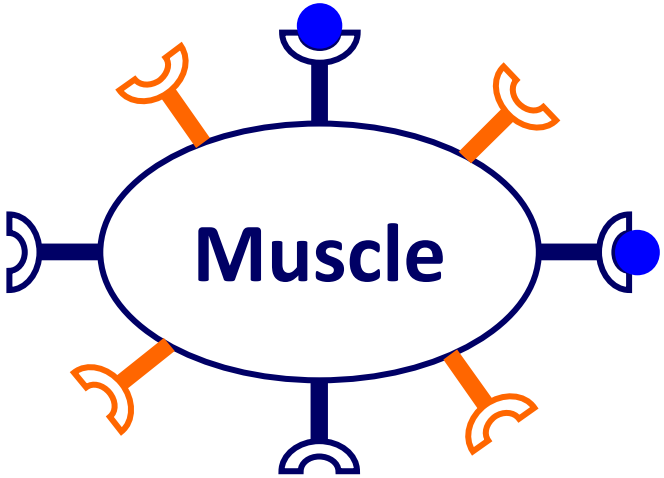




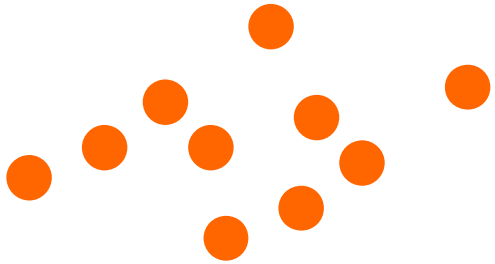
Myostatin inhibition

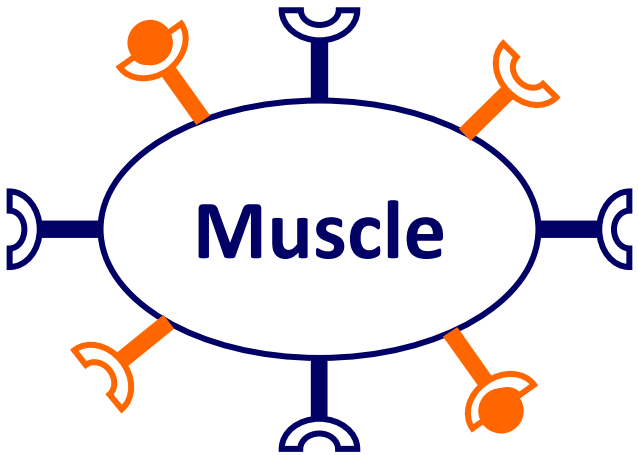
Muscle growth factors





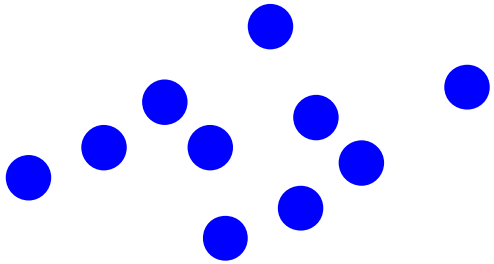
Muscle growth inhibitors



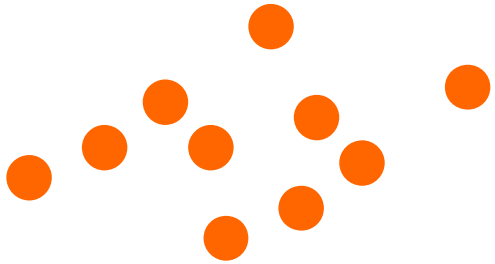


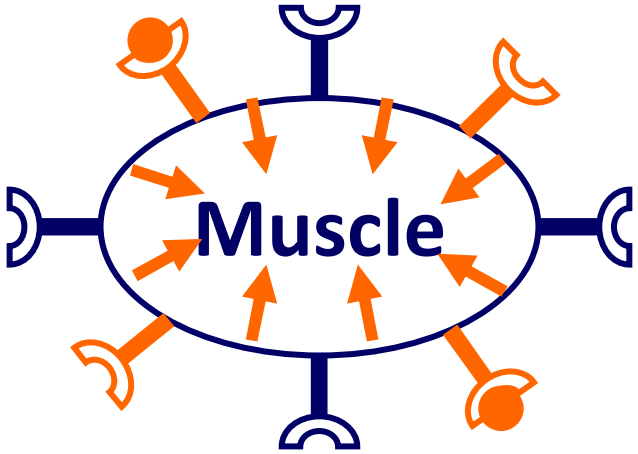
Myostatin inhibition

Muscle growth factors



Muscle growth inhibitors



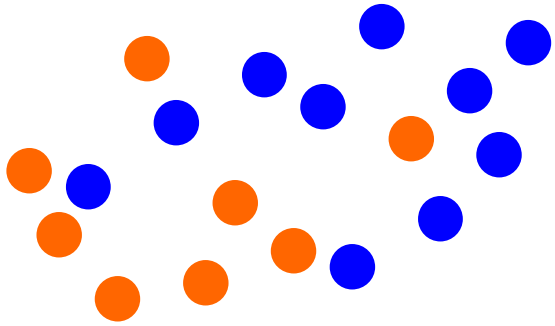


Myostatin inhibition

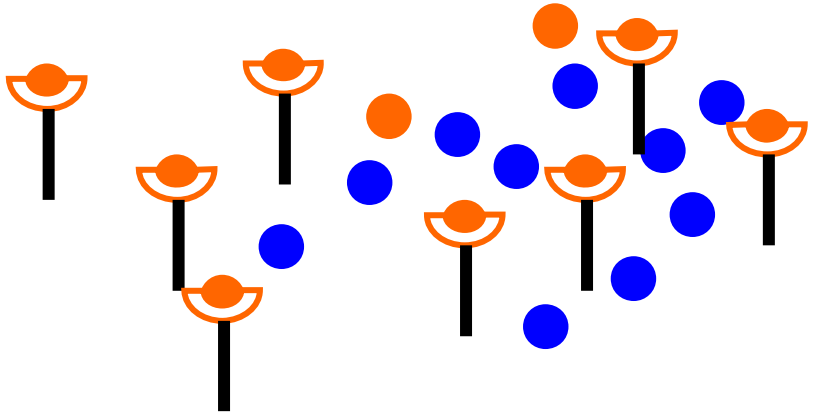
- Myostatine inhibits muscle growth
- Animals/human without myostatin: muscular!
- Inhibit myostatin → increase muscle size
- Compensate loss of muscle in patients

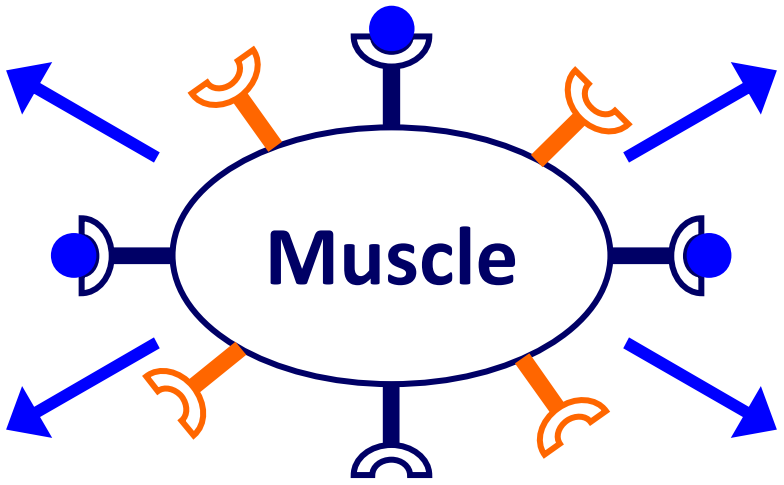
Myostatin inhibition

Normal



Myostatin inhibition





Myostatin inhibition

Acceloron generated potent myostatin antibody

Tested in healthy mouse

- More muscle and less fat

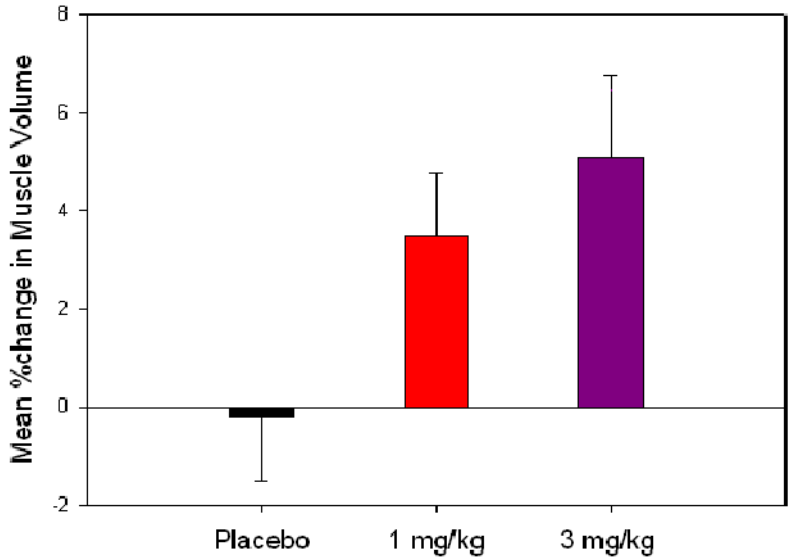
Mdx mice

- More muscle and less fat
- Stronger muscle

Myostatin inhibition

Tested in healthy volunteers

- Well tolerated
- Increased muscle mass
- 1 kg in 2 weeks for highest dose (5% increase!)
- Multiple doses also well tolerated



Summary/Outlook

- Lack of dystrophin underlies problems in Duchenne patients
- Therapies aim to tackle one or more of these problems
- Lot is known about disease → primarily due to research funded by patient advocacy group
- Due to improved care life expectancy is increased from ~16 to ~30!