LEIDEN UNIVERSITY MEDICAL CENTER

Opportunities and challenges of therapeutics approaches for DMD

Annemieke Aartsma-Rus

January 12 2014

Overview

- What does dystrophin do?
- What happens when there is no dystrophin?
- How do different therapeutic approaches aim to prevent/delay these processes?
- For each approach
  - State of the art
  - Opportunities
  - Challenges
- Exon skipping discussed in seperate talk

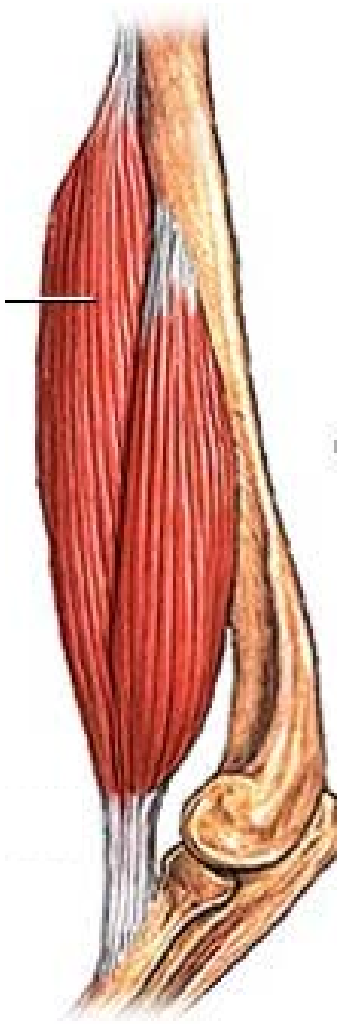
Some basic biology: genes & proteins

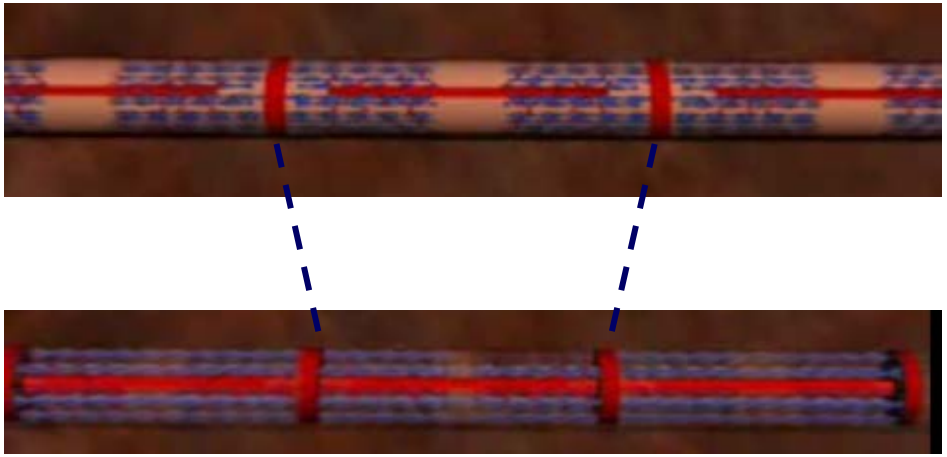
- Proteins are building blocks of our body
- Genes contain blueprint for proteins
- Mistake in gene → mistake in protein
- Genes have a volume switch
  (protein only produced in proper tissue)
- Dystrophin protein has a function in muscle
- Mistake in dystrophin → Problems in muscle

Muscles

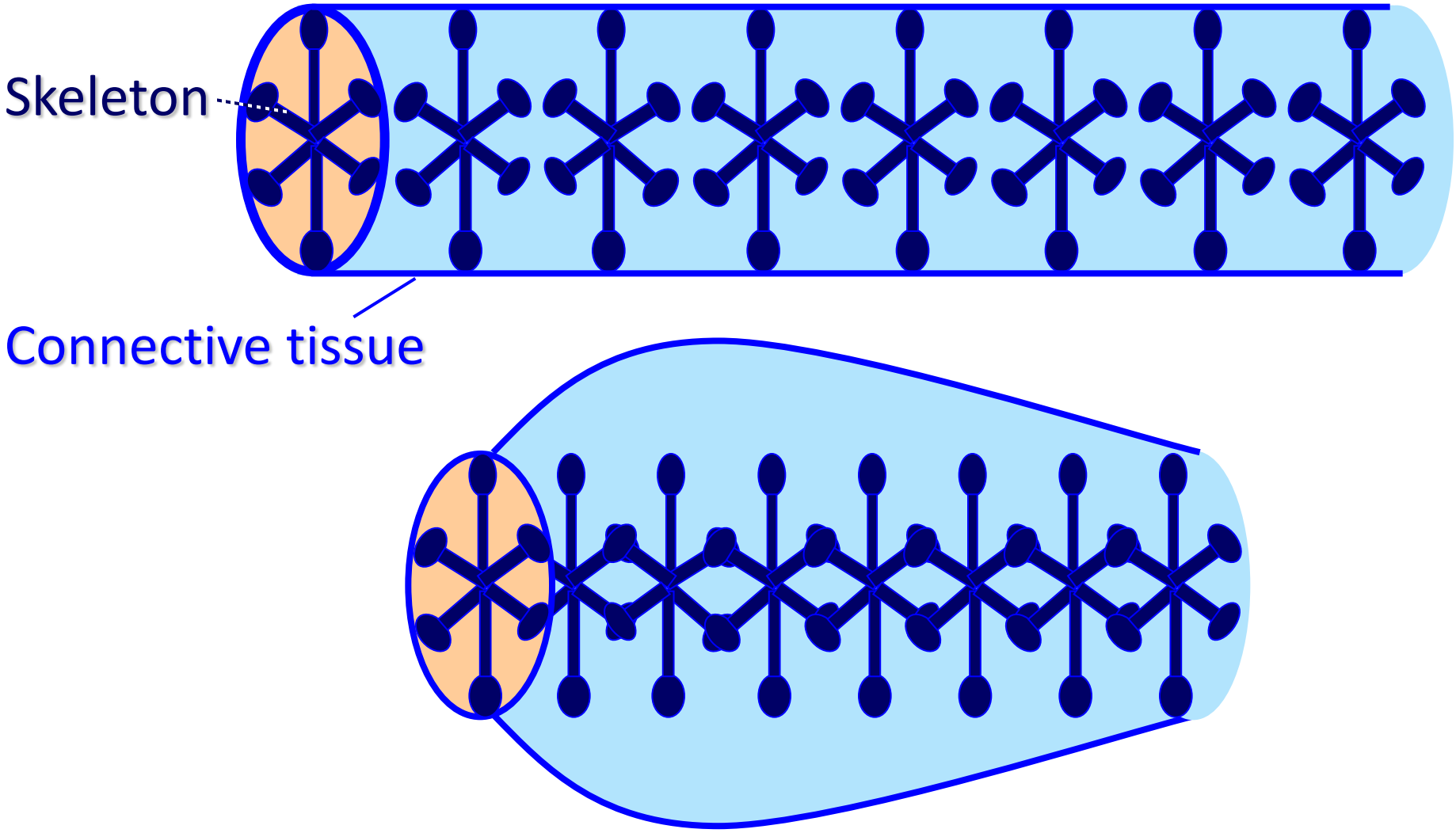
- 30-40% of our body is muscle
- >750 different muscles
- Muscles can grow bigger or smaller
- Muscles use a lot of energy
- Only maintained when needed
- Muscles are damaged when used too much
- Muscles have efficient system to repair damage and prevent future damage (grow bigger)

Muscle contraction





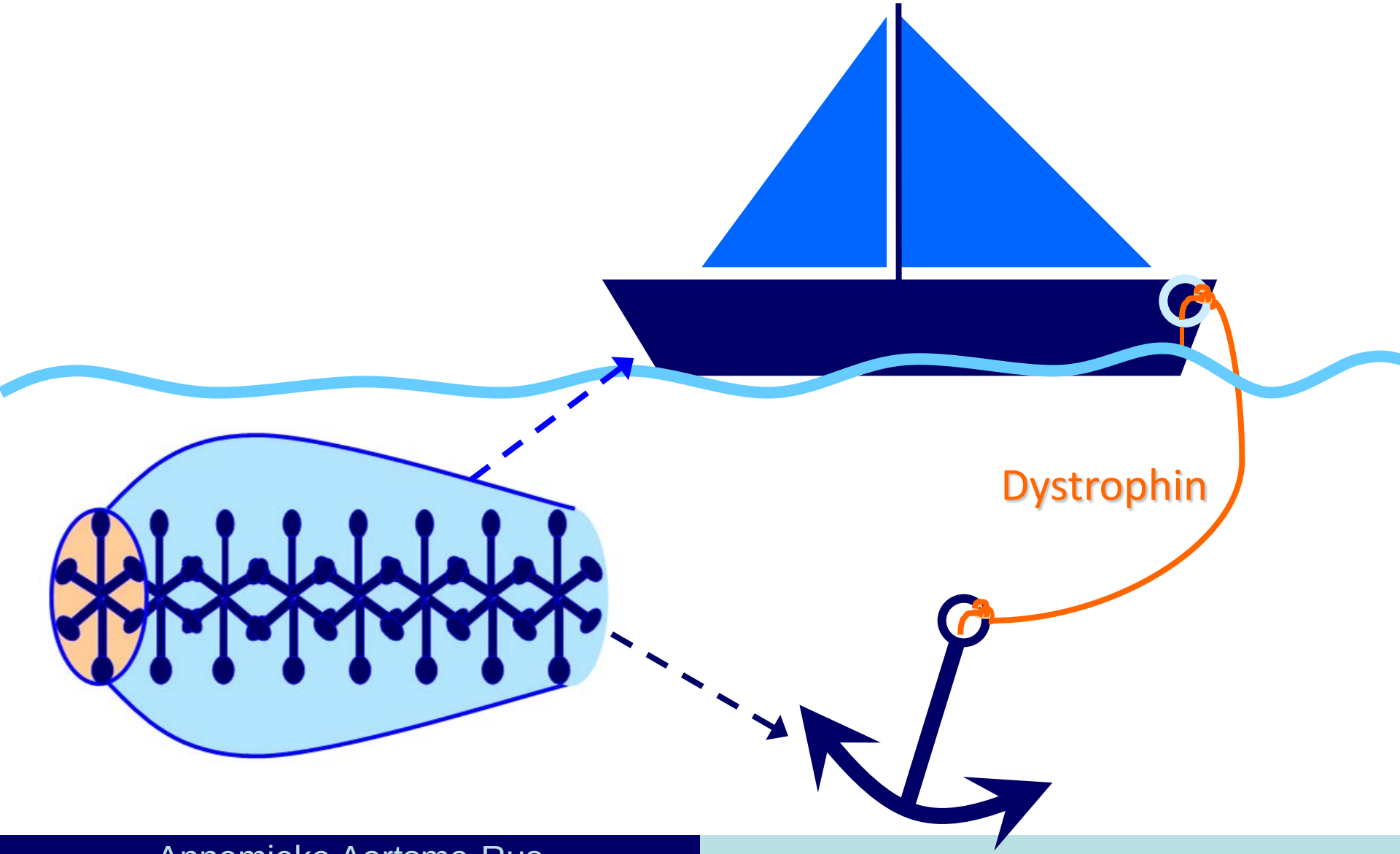
Muscle fibers



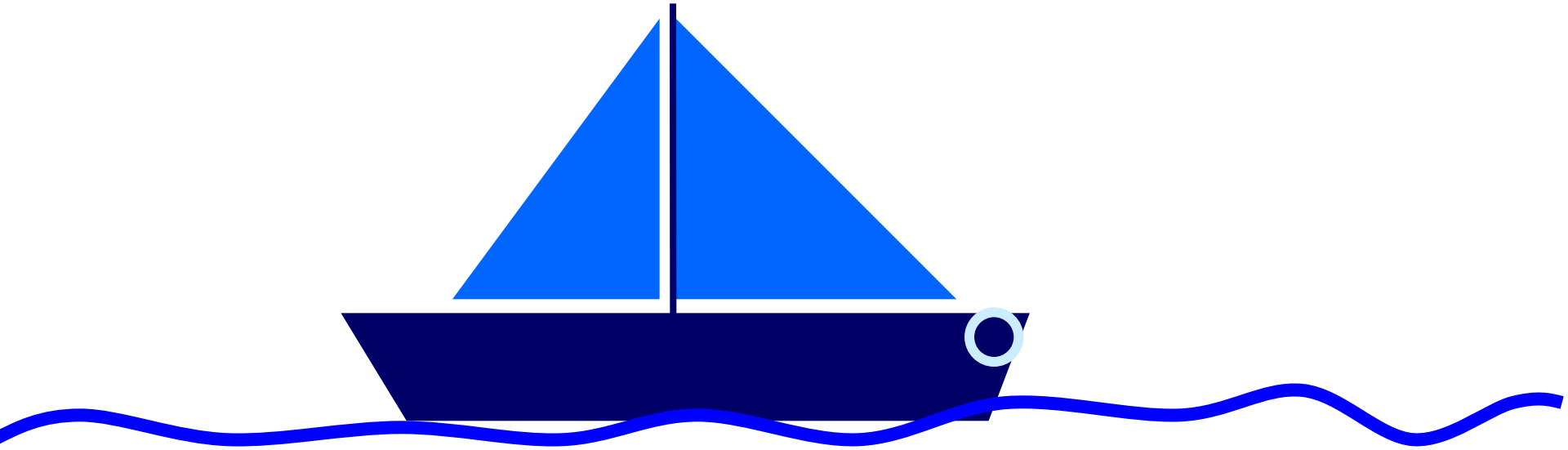
Dystrophin

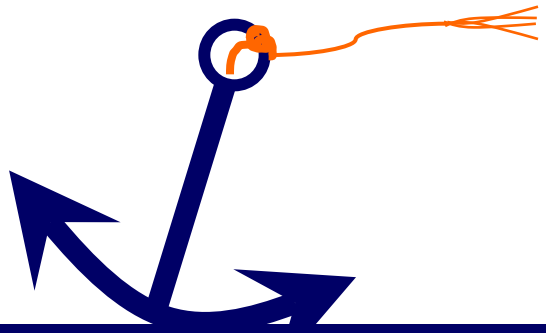
- Dystrophin provides stability to muscle fibers during contraction
- Connects skeleton of muscle fibers to connective tissues surrounding muscle fibers
- No dystrophin → Connection lost
- Muscle more sensitive to damage
- Chronic damage: repair system cannot keep up
- Loss of muscle tissue and function

Dystrophin

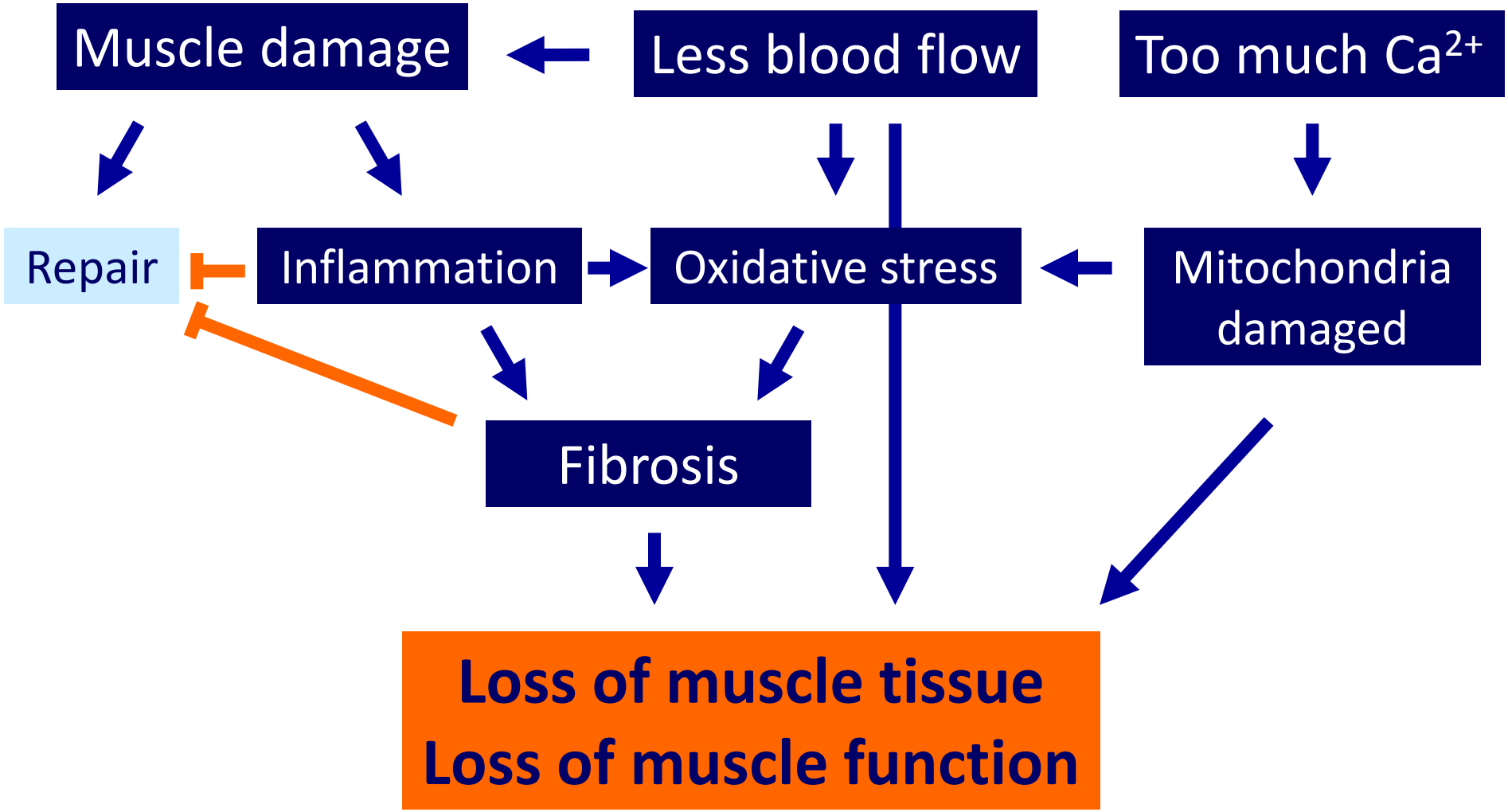


Duchenne: no functional dystrophin





No functional dystrophin

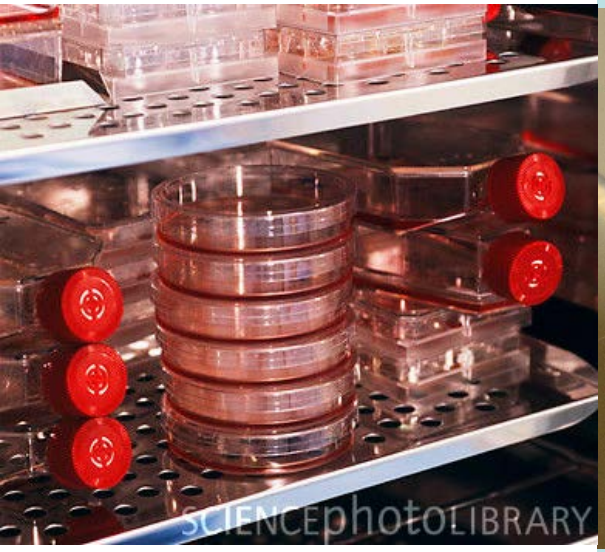


Therapeutic options

- Drug Therapy
  - Anti-inflammatory
  - Anti-fibrosis/anti-oxidants
  - Improve blood-flow
  - Improve muscle mass
  - Utrophin upregulation
- Cell therapy
- Genetic therapy
  - Gene therapy
  - Ataluren (stop codon readthrough)
  - Exon skipping

Therapeutic development

Cultured Cells



- First test
- Feasibility
- Small numbers
- No circulation
- No immunity
- No organs

Animal models

- *Mdx* mouse
- No dystrophin
- Organs, immunity

Limitation

- Regenerates well
- High metabolism

Patients

Phase 1/2

- Safety
- No control group

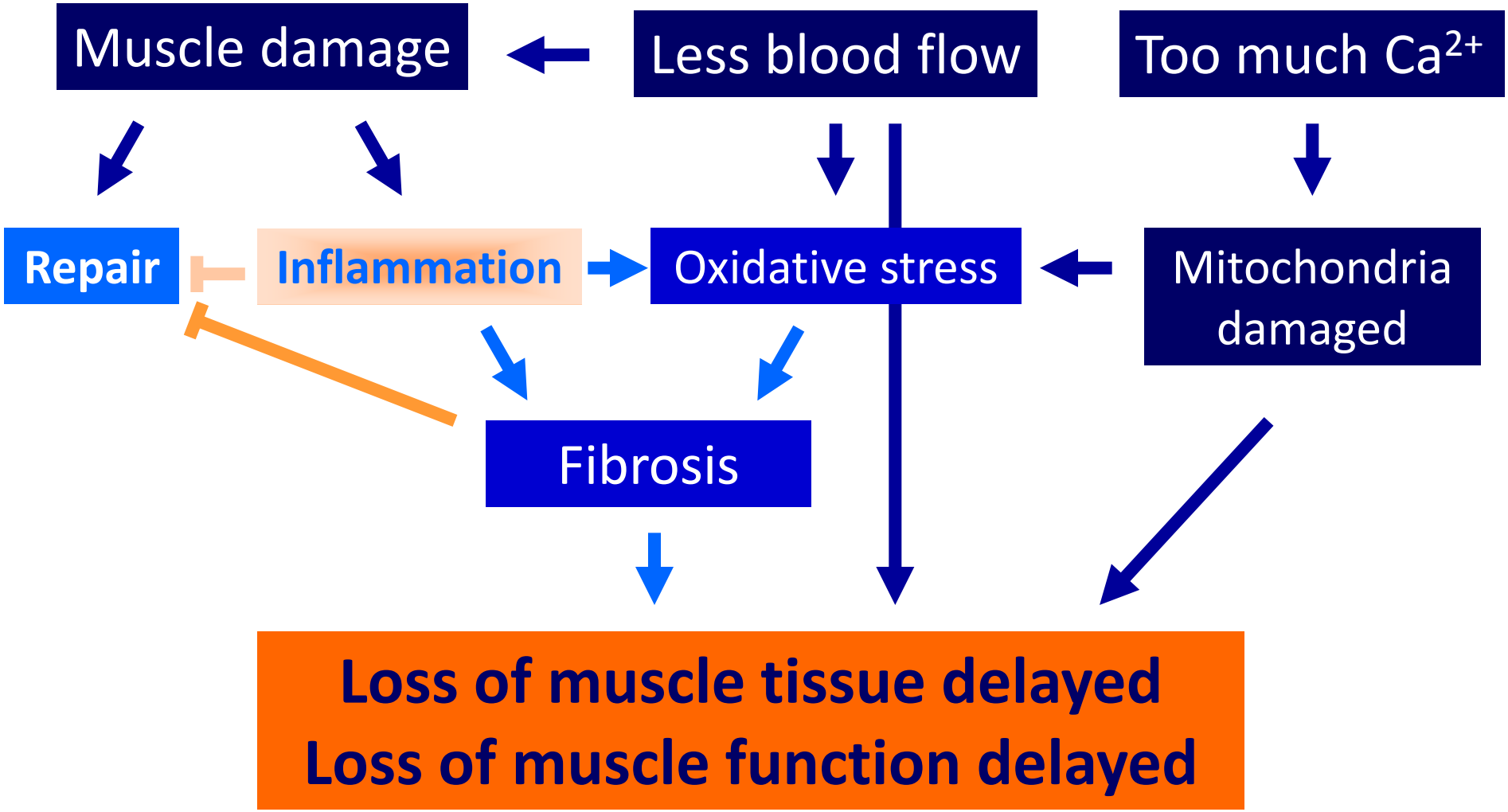
Phase 2-3:

- Effective?
- Long term safety?

Development of therapies

- Tests from cell and animal models to clinical trials
- All steps are important to show proof-of-concept (does it work in a model system ?)
- Next steps are always more complicated
- Success in one step is no guarantee for success in subsequent steps
- Clinical trials are experiments in humans
  - May not work, may not be safe

Anti-inflammatory drugs



Anti-inflammatory drugs

- Prednisone and deflazocort used most often
- Benefits and side effects
- Treatment regimens not standardized: FOR-DMD

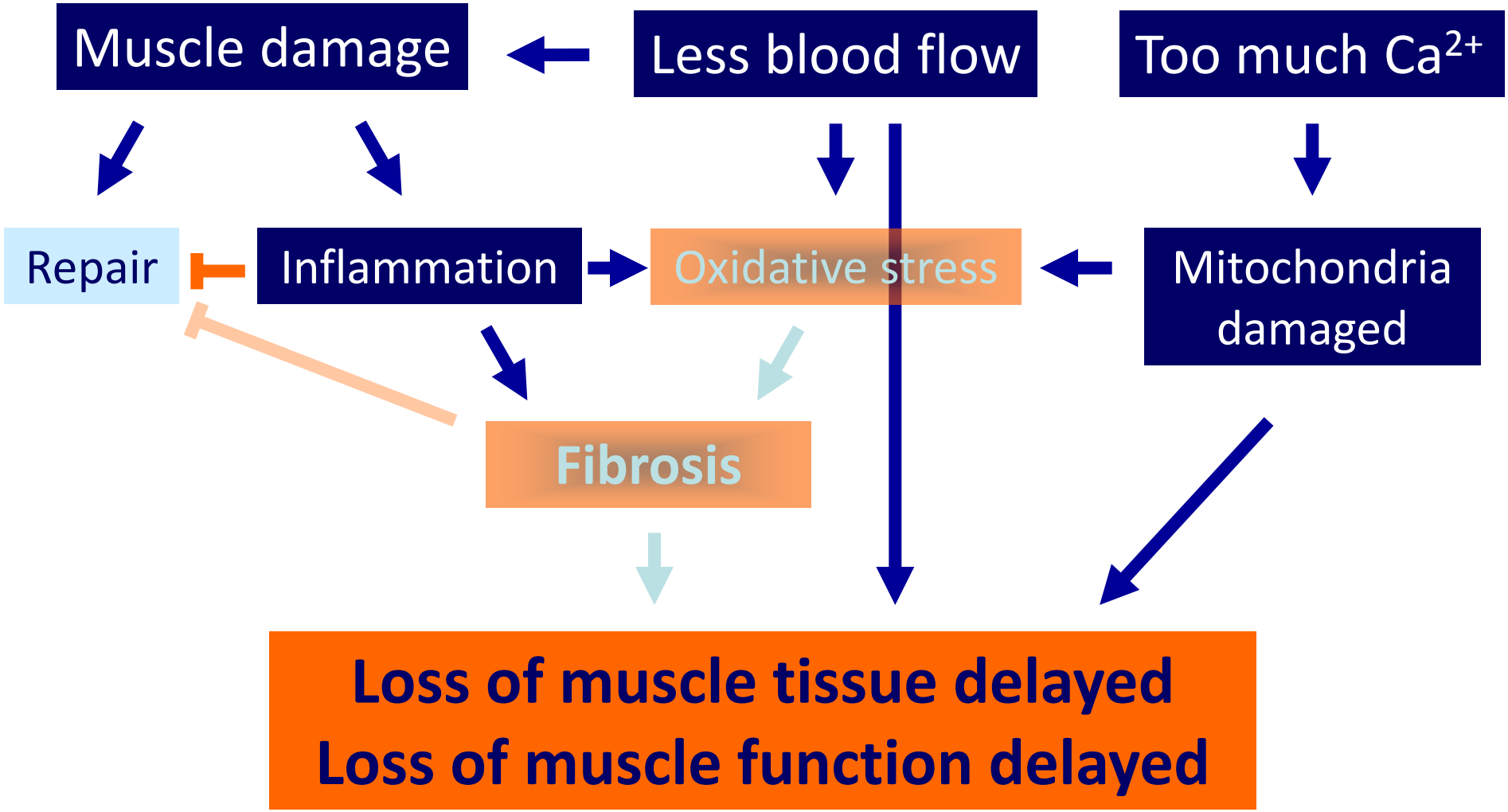
Alternative anti-inflammatory drugs

- Cyclosporine

  Tested in clinical trial but no effect
- CAT1000 (Catabasis) and VBP15 (ReveraGen)

  Preclinical development

Anti-fibrosis/anti-oxidants



Anti-fibrosis/anti-oxidants

Idebenone (Santhera)

- Anti-oxidant
- Reduces fibrosis in mouse models
- Phase II trial completed (safety trial)
- Phase III trial ongoing
    - Stage 1: Corticosteroid naieve patients
    - Stage 2: Patients on cortocosteroids

Anti-fibrosis/anti-oxidants

Green tea extract (EGCG)

- Anti-oxidant
- Reduces fibrosis in mouse models
- Trial ongoing in Germany in patients

Anti-fibrosis/anti-oxidants

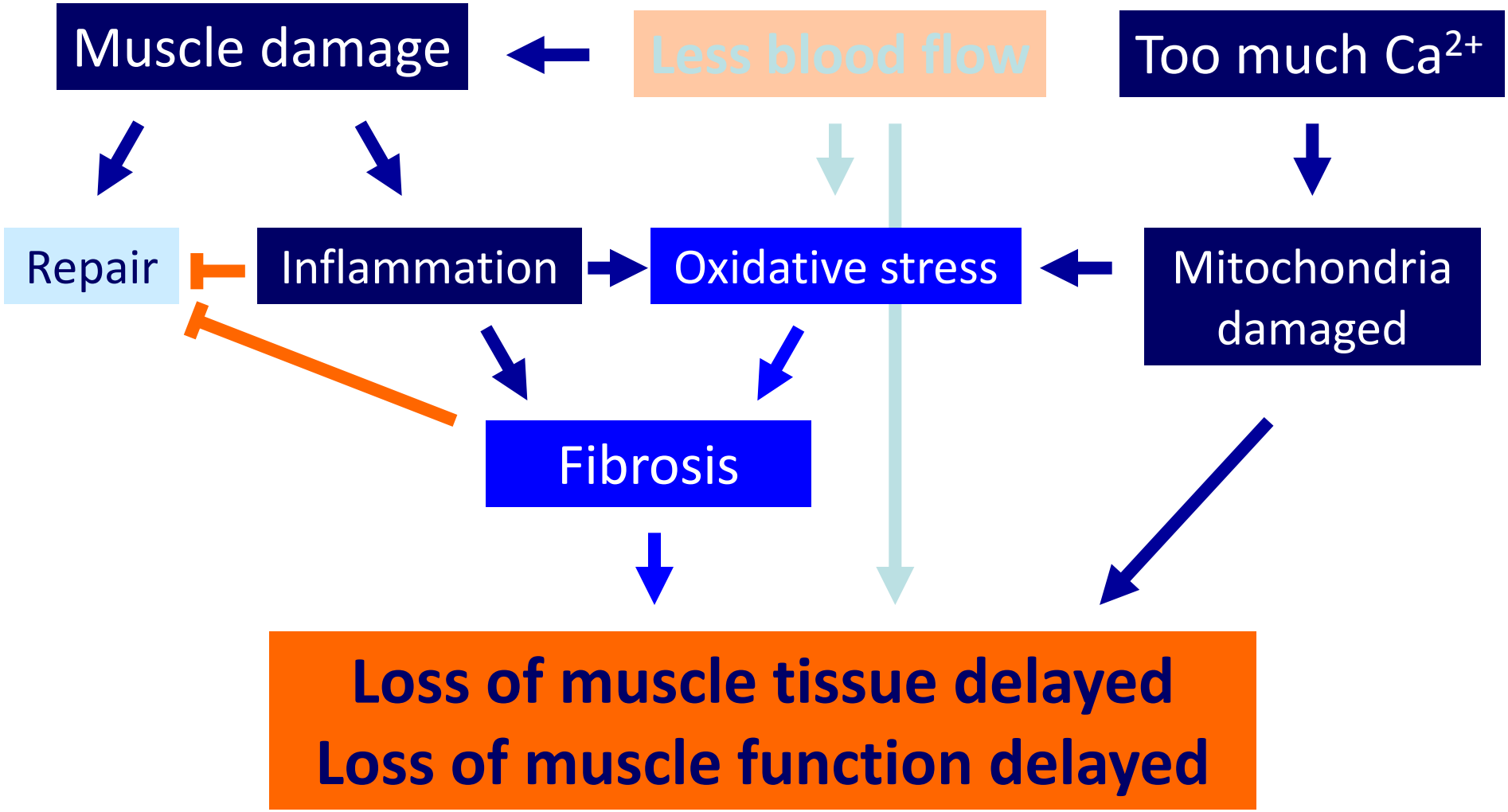
Pentoxifylline

- Reduces fibrosis in mouse models
- Trial completed
  - Poor toleration by patients (nausea)

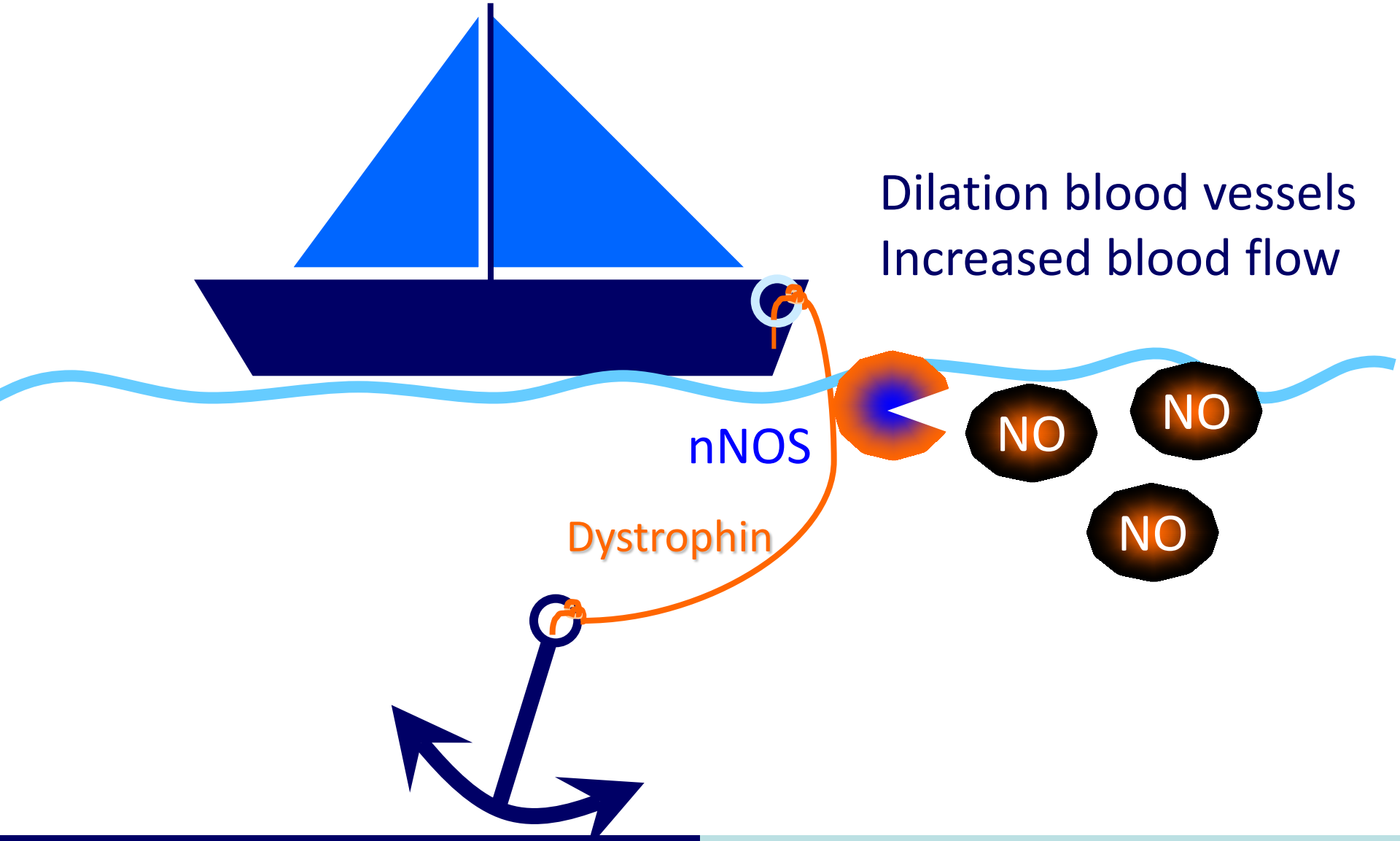
HT-100 (halifuginone) and tamoxifen

- Tested in preclinical studies (animal models)

Vasodilators

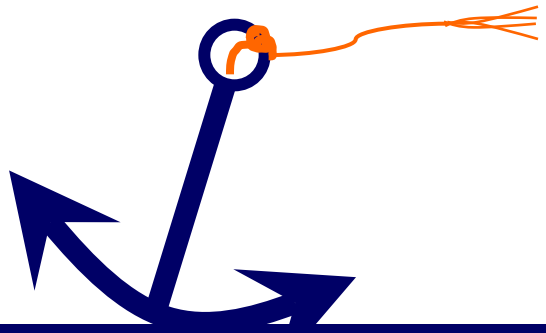


nNOS and dystrophin

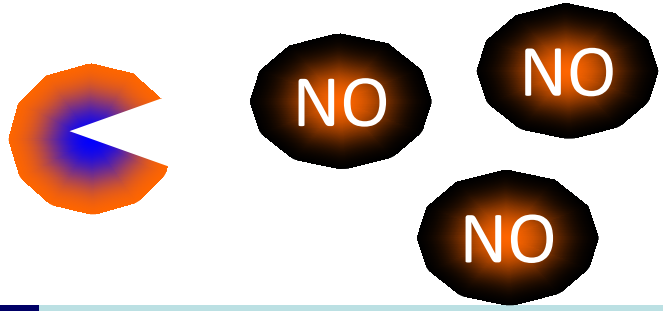


nNOS and dystrophin

No dilation of blood vessels



Oxidative stress



Vasodilators

ACE-inhibitors (e.g. lisinopril)

- Angiotensin 2 leads to vasoconstriction
- ACE: angiotensin 1 ➔ angiotensin 2
- Trials ongoing to test effect on heart function

  USA, Canada and Japan

Vasodilators

PDE5-inhibitors (e.g. sildenafil and tadalafil)

- PDE enzymes counteract NO cascade
- Inhibit inhibitor: prolong effect of dilation

Sildenafil (viagra)

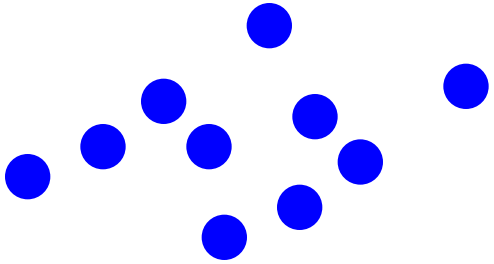
- Trial suspended (USA)

Tadalafil

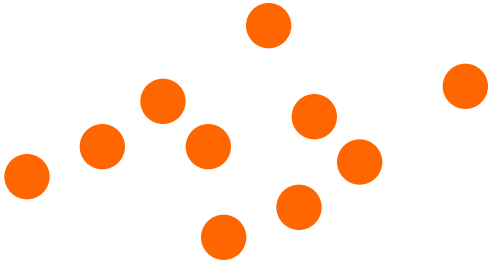
- Promising results in Becker patients
- Global trial coordinated by Eli Lily

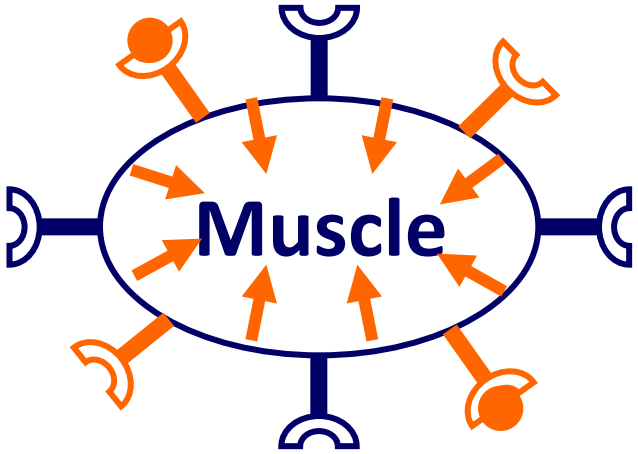
Improve muscle mass

Muscle growth factors



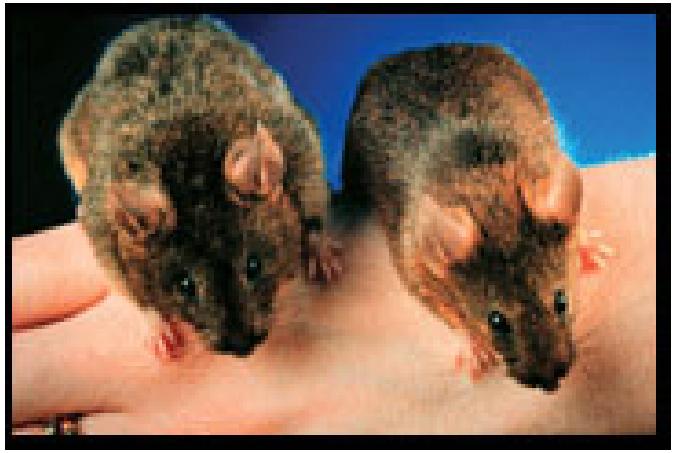
Muscle growth inhibitors





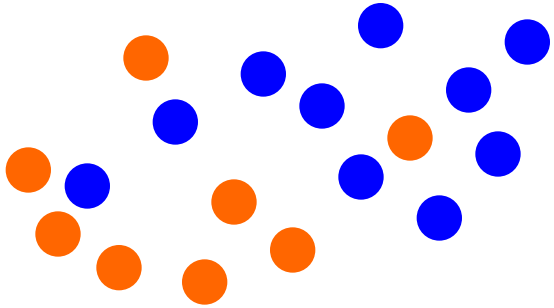
Myostatin inhibition

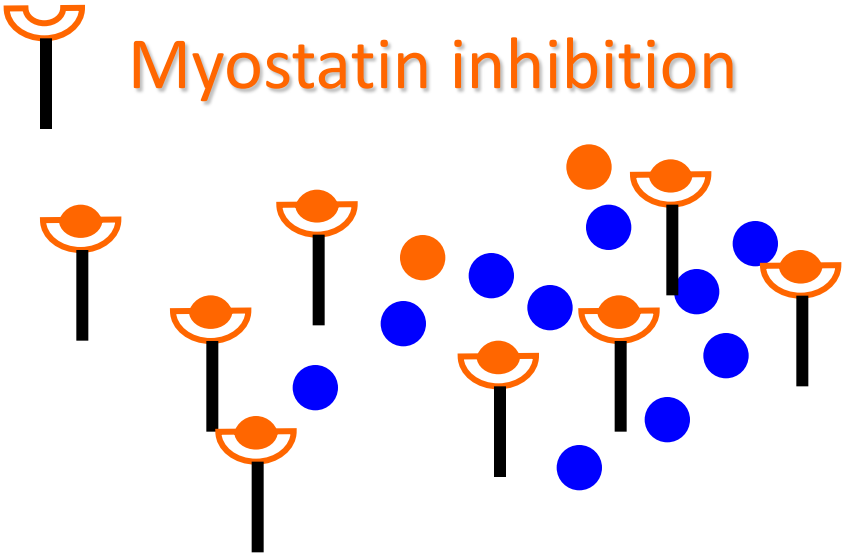
- Myostatin inhibits muscle growth
- Animals/humans without myostatin: muscular
- Inhibit myostatin ➔ larger muscle
- Compensate loss of muscle for DMD patients?

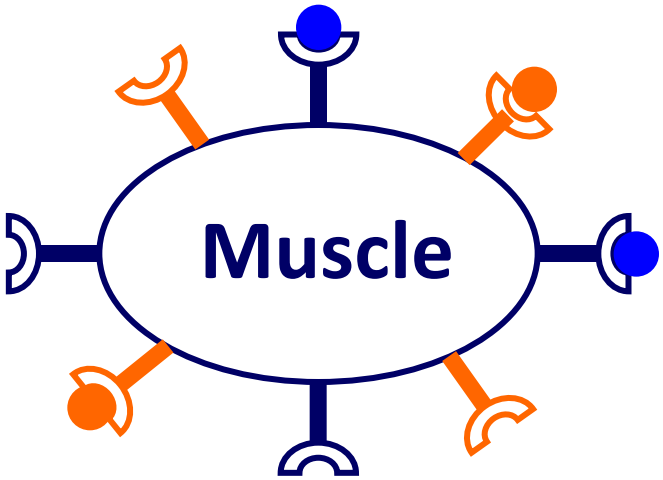


Myostatin inhibition

Normal







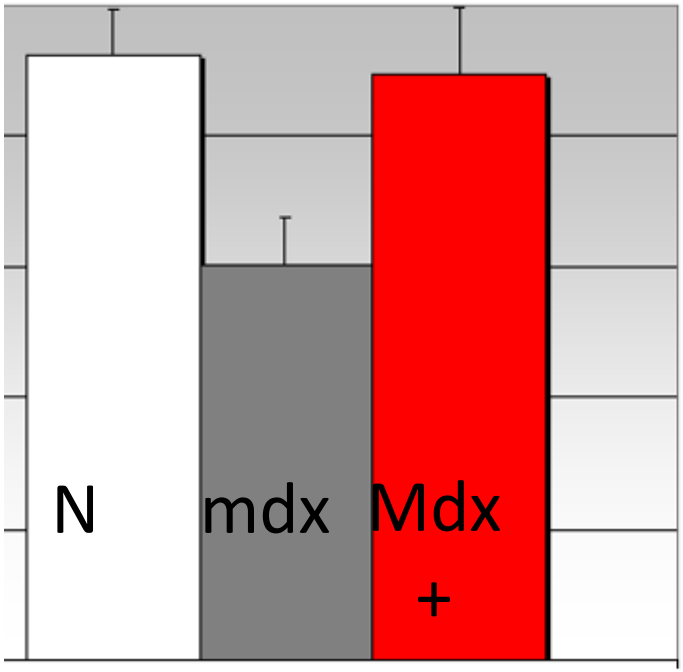
Myostatin inhibition

Myo-29 antibody tested in LGMD and BMD patients

- Safe
- No effect on muscle mass
- Short trial
- Longer trial ongoing (Pfizer)

Accelleron antibody (ACE-031)

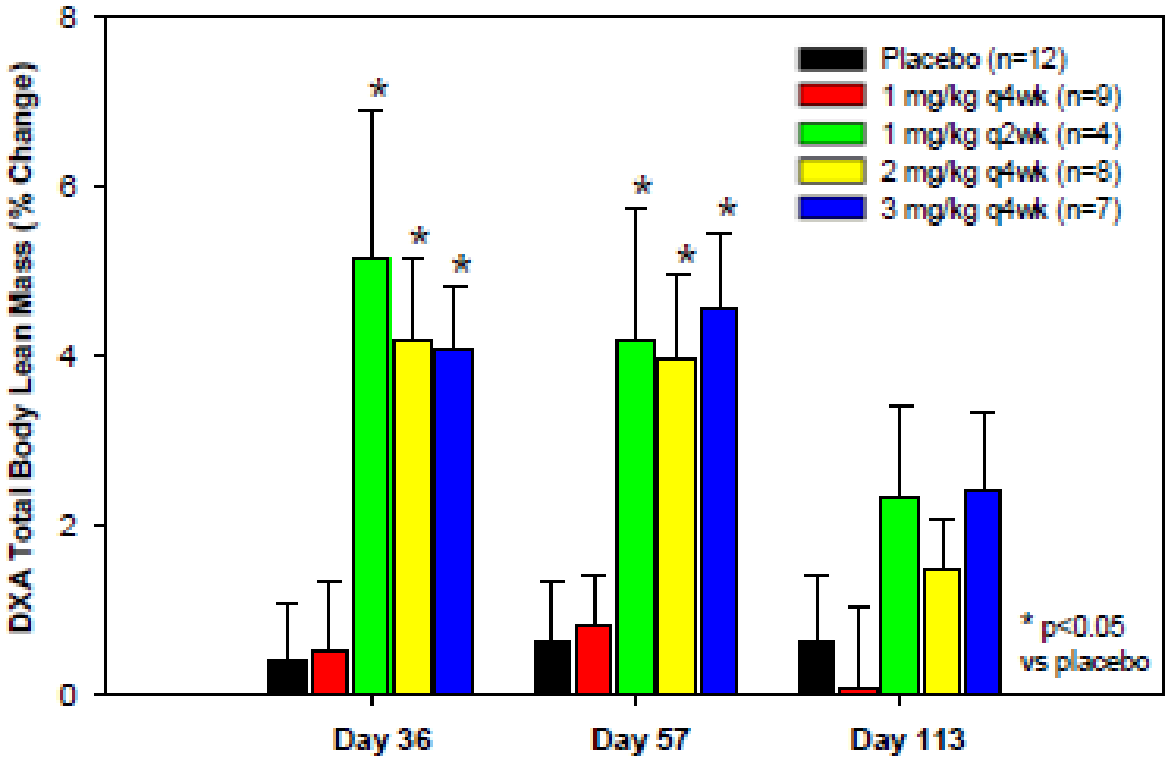
- More muscle
- Less fat
- *Mdx*: stronger muscle



Myostatin inhibition

Tested in healthy volunteers

- Well tolerated, also after repeated injections
- More muscle mass, less fat



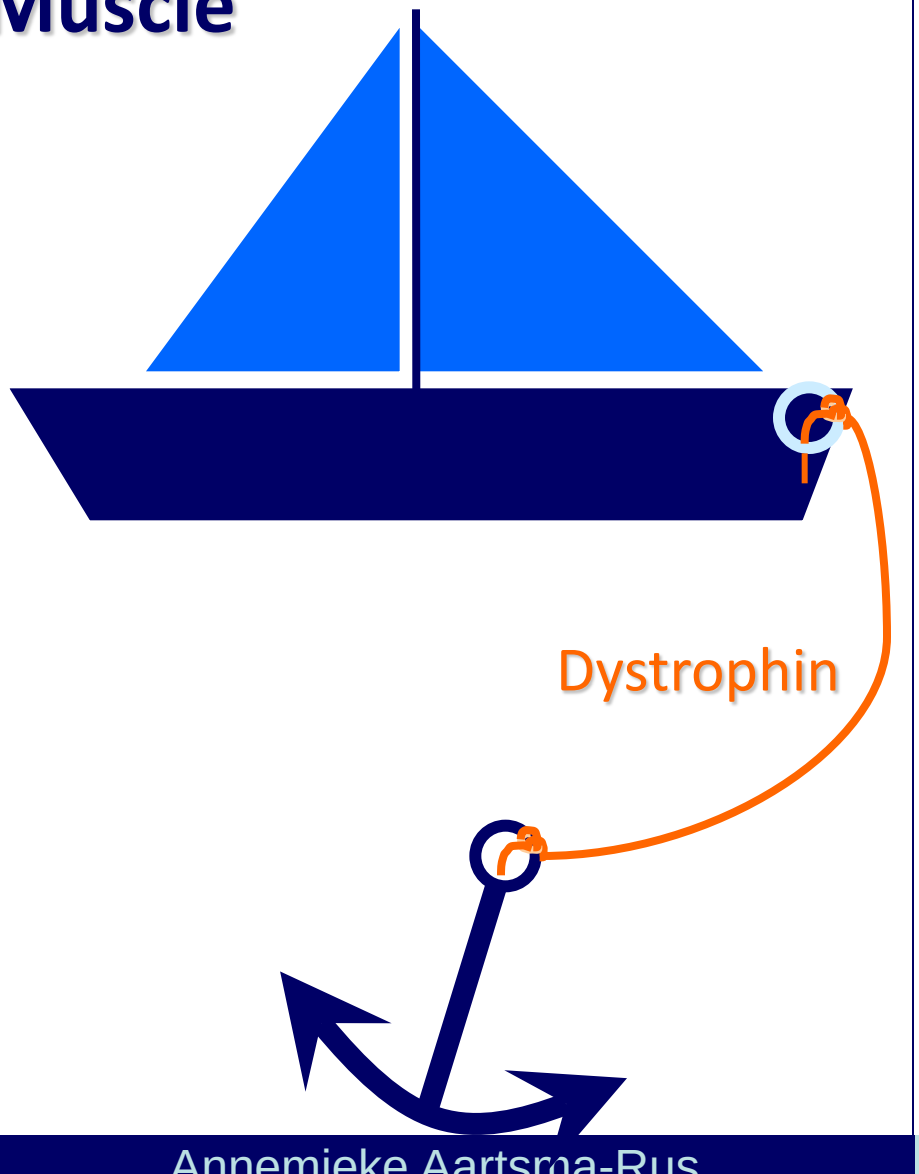
Myostatin inhibition

Clinical trial in DMD patients (Canada)

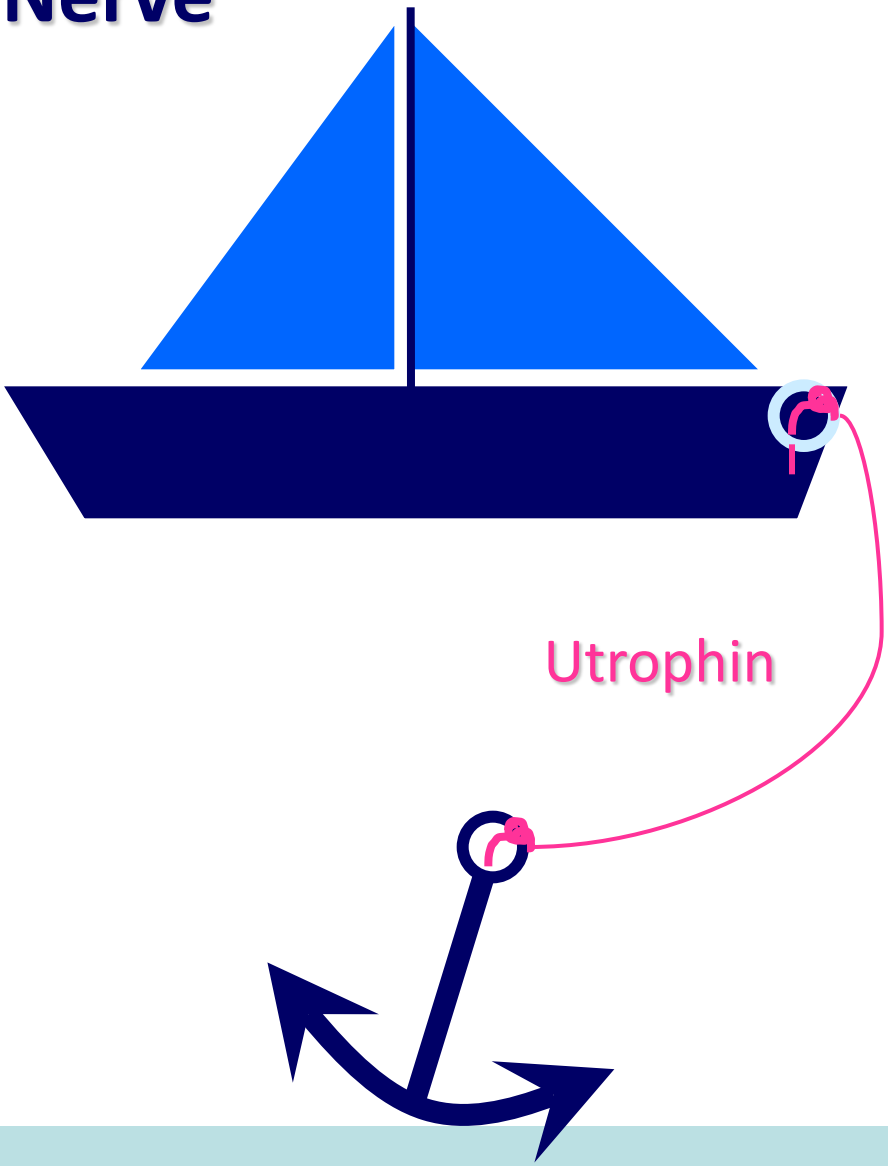
- 12 week treatment, 3 doses
- Placebo group (1:3 placebo)
- Test for safety and finding optimal dose
- Clinical trial terminated!
- Some patients had nose and gum bleeds
- Due to off-target effect antibody
- Development terminated
- Pfizer developing more specific antibody

Utrophin upregulation

Muscle

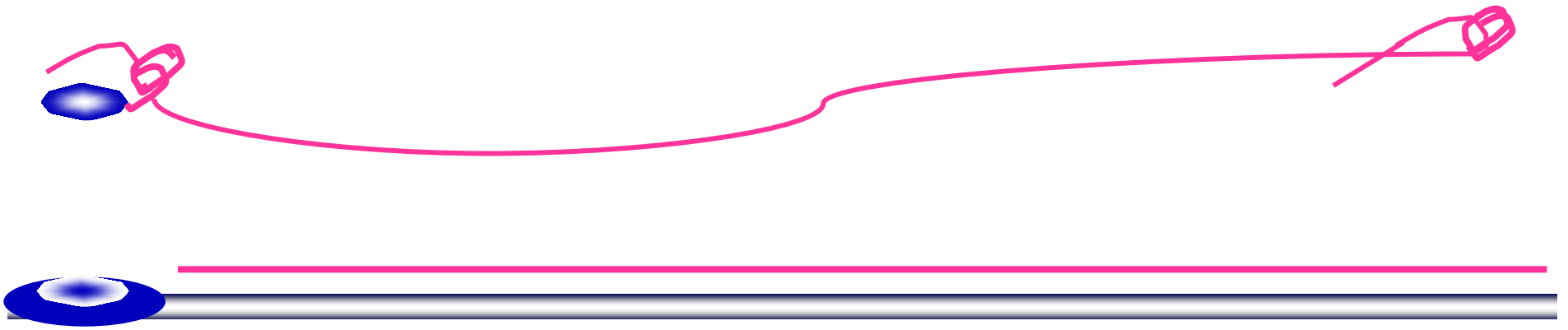


Nerve



Utrophin upregulation

- Utrophin resembles dystrophin
- Utrophin can take over dystrophin function
- Expressed in nerve cells, hardly in muscle
- Find ways to get more utrophin in muscle



Utrophin gene volume switch

Utrophin upregulation

- Find compound to switch on utrophin gene volume
- High throughput screening in cell models
- Potential drugs screened further in patient-derived cell cultures and mouse models
- Candidate drug tested in healthy volunteers (BMN195/SMNT C1100) by Biomarine
- Uptake not sufficient
- Summit made new formulation
- Healthy volunteers: improved uptake!

Utrophin vs. Dystrophin

- Similar proteins, but not identical
- Utrophin can take over many dystrophin functions
- Utrophin does not recruit nNOS
- Utrophin does not recruit energy producing organels (mitochondria)

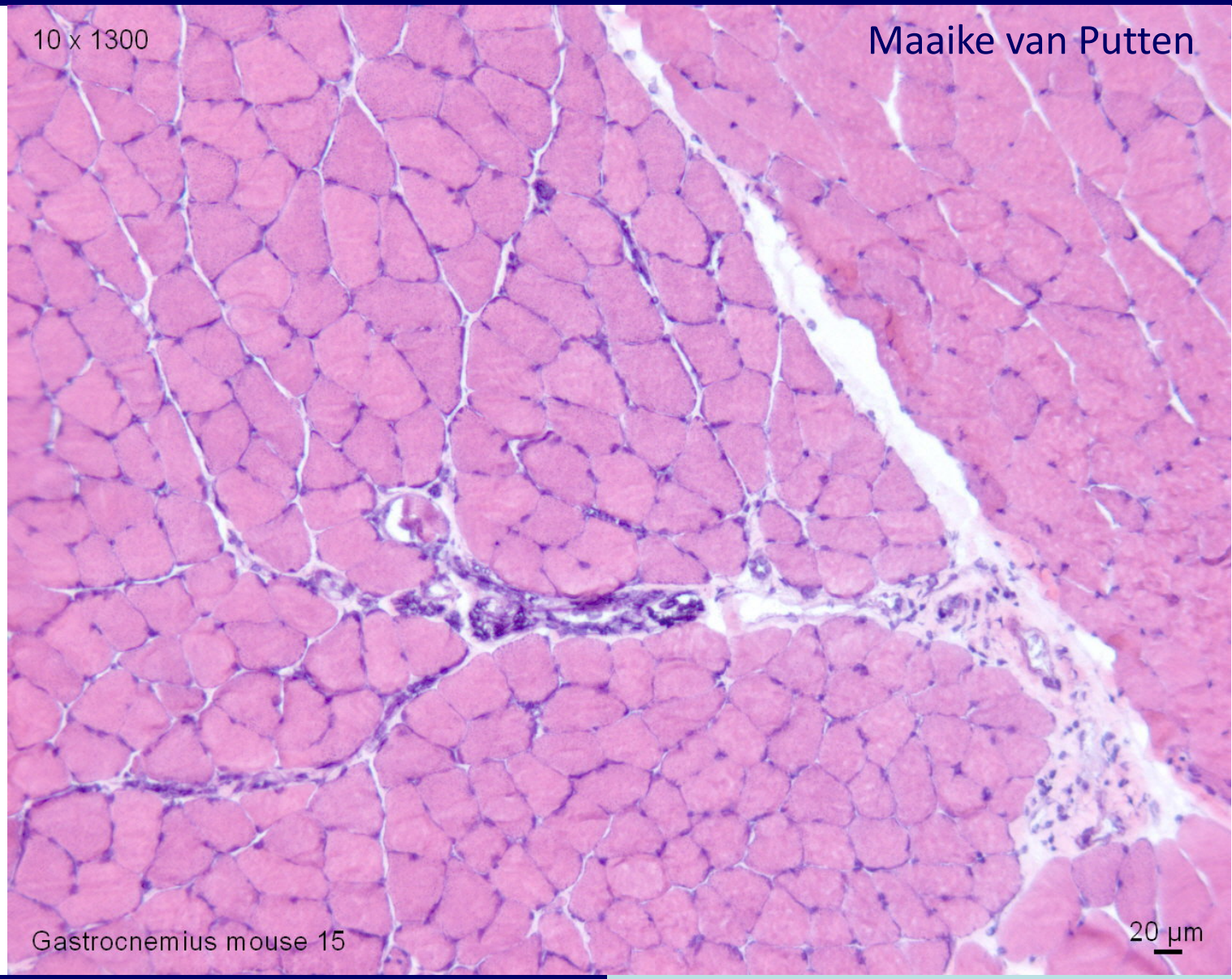
Utrophin upregulation summary

- Opportunities
  - Applicable to all patients
  - Oral drugs
- Challenges
  - Utrophin cannot fully compensate for all dystrophin functions

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?

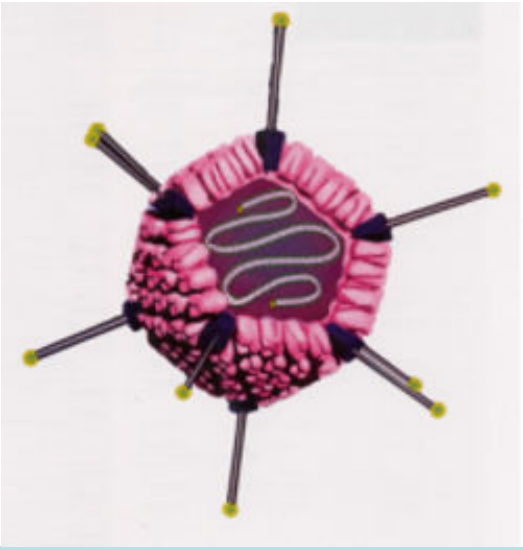
Gene Therapy



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)



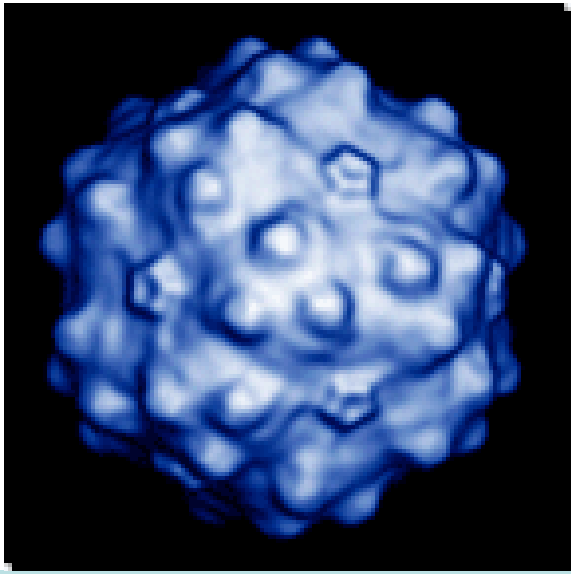
Gene Therapy

Which virus?

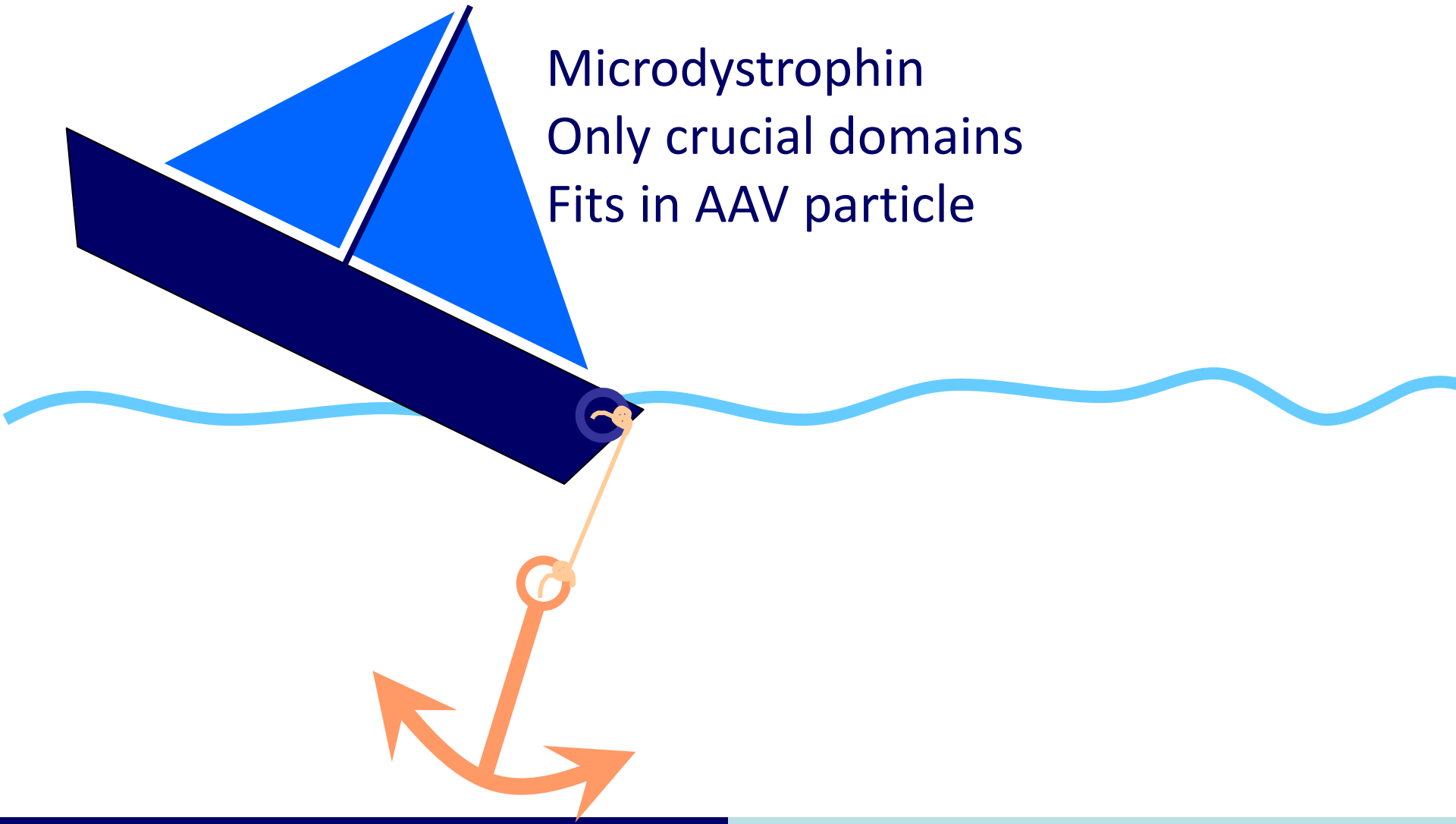
- Most viruses do not infect muscle tissue
  - Muscle cells do not divide often
  - Lot of connective tissue (filters out viruses)
- Exception: adeno-associated virus (AAV)
- Preference for muscle
- Not pathogenic in man

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



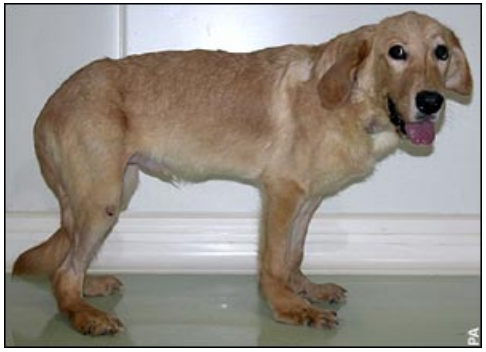
Gene Therapy



Microdystrophin
Only crucial domains
Fits in AAV particle

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

Upscaling

- Mouse — 12 gram muscle
- Monkey — 4 kg muscle
- Human boy — 10-25 kg muscle

333x

2-6x

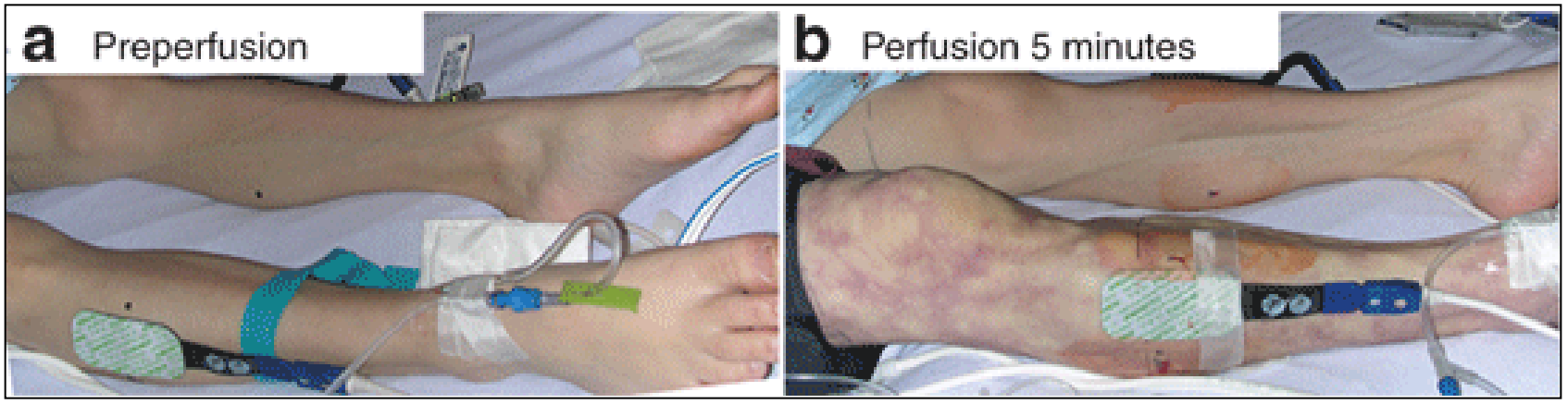
- Monkeys and humans much larger than mice
- Need much more viruses
- Manufacturing systems optimized to allow production of sufficient amounts for treating human limbs at clinical grade

Delivery

- Whole animal delivery possible for mouse
  - Not feasible (yet) for large animals
  - Limited by amount of virus
    - Produced
    - Injected
- Whole limb delivery in development for human
  - Hydrodynamic limb perfusion (most efficient)
  - Regional limb perfusion (less damage)

Limb perfusion

- Tested in monkeys and dogs with ‘color gene’
  - Delivery to multiple muscles feasible
- Tested in adult MD patients with saline
- Possible for lower leg or arm (less efficient)



- Not yet tested in humans to deliver gene

Gene Therapy

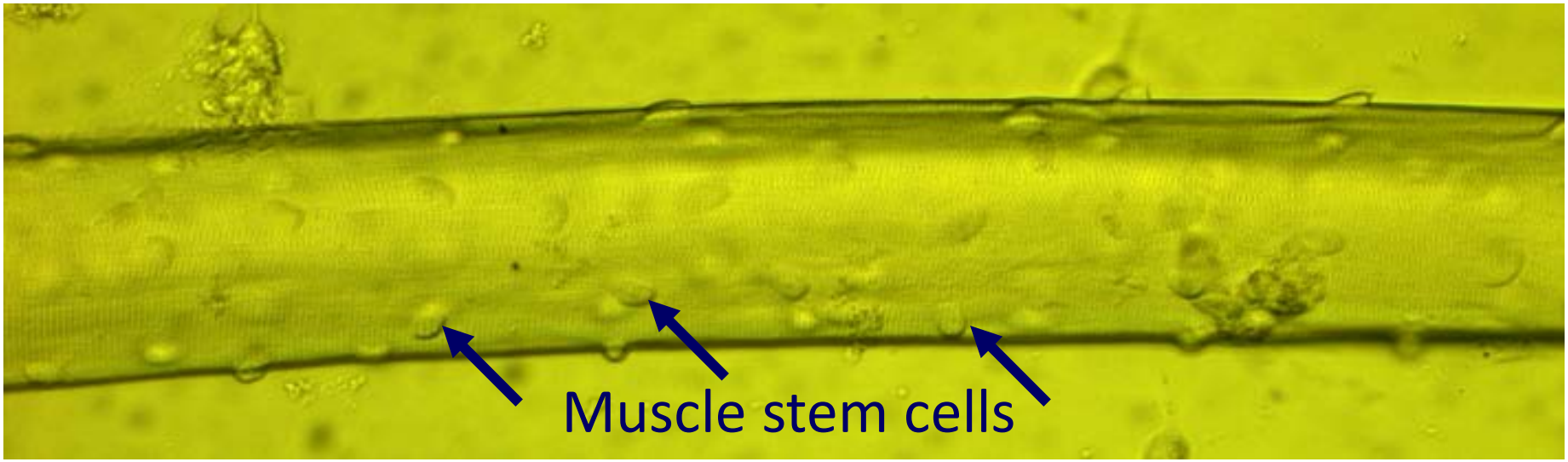
Immune problem prohibits multiple treatments

- Other AAV subtypes may not be recognized by immune system
- Immune suppression
  - Only before and immediately after treatment?
- Use only DNA (Jon Wolff, France)
  - But efficiency is lower than when using virus

Gene Therapy Summary

- Opportunities
  - Applicable to all patients
- Currently in early clincial development (safety/tolerability tests)
- Challenges
  - Microdystrophin only partially functional
  - Delivery
  - Immunity

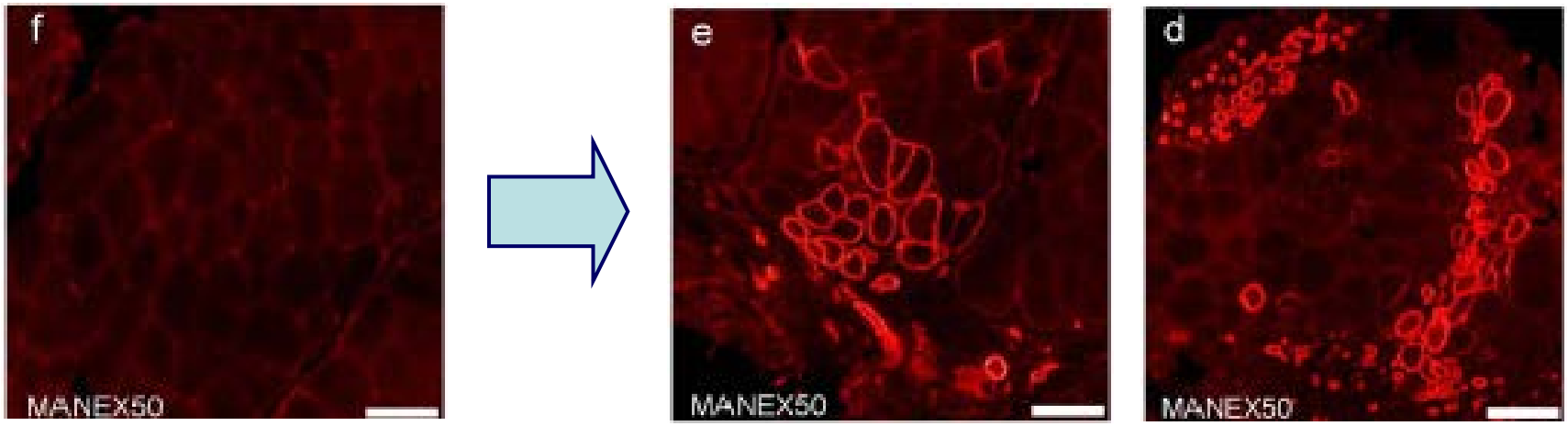
Cell therapy



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

Muscle stem cells (myoblasts)

- Immune response (suppress)
- Do not exit circulation after injection
- Local injection: stay close to injection site
- Tremblay (Canada): multiple local injections
  - Local dystrophin restoration
  - Not feasible for larger muscles



Stem cell therapy

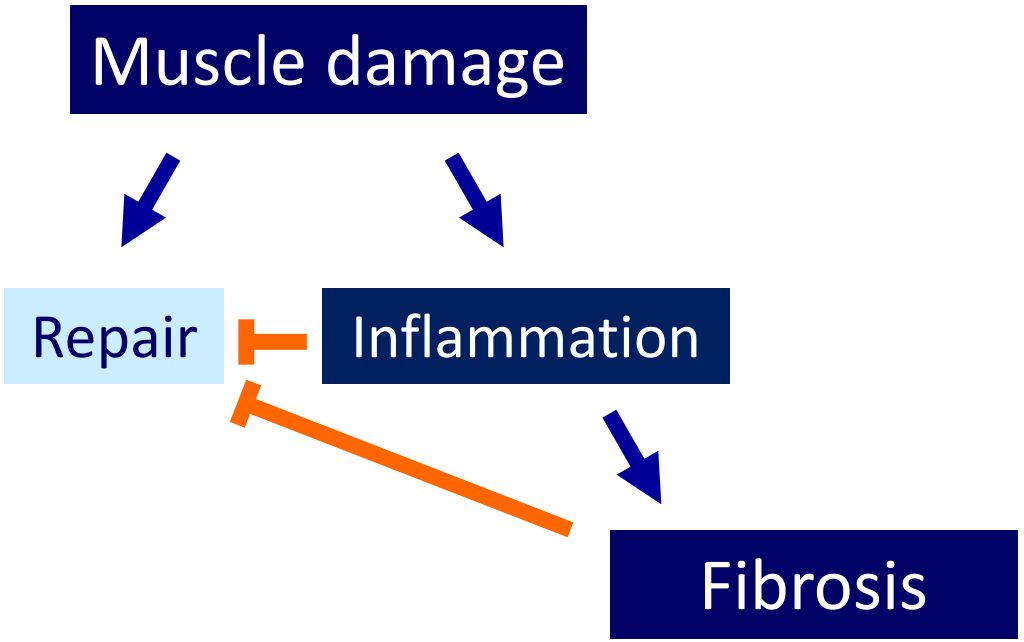
Stem cells from fat, bone and bloodvessel walls

- Can exit bloodstream and migrate into muscle
- Very low efficiency

Mesangioblasts most promising

- Encouraging results in dog model
- Safety trial ongoing in Italy (Giulio Cossu)
- 3 patients received stem cells
- Some side effects
- Preparing for injection 2 more patients

Niche



- Dystrophic muscle is damaged (scar tissue/fibrosis)
- The few transplanted stem cells that reach muscle
  - Do not receive proper signals to become muscle
  - Receive signals from scar tissue: more fibrosis

Immunity

- Transplanting cells from one person to another will elicit an immune response
- Need chronic immune suppression
  - Side effects
- Isolate patient stem cells, expand in the lab, correct mutation with gene therapy & transplant
  - No immune response
  - Gene therapy more efficient in cultured cells than in muscles

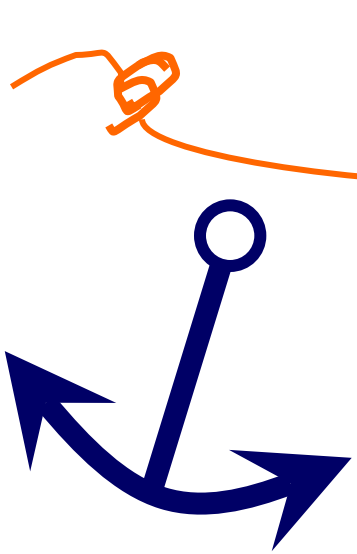
Cell therapy summary

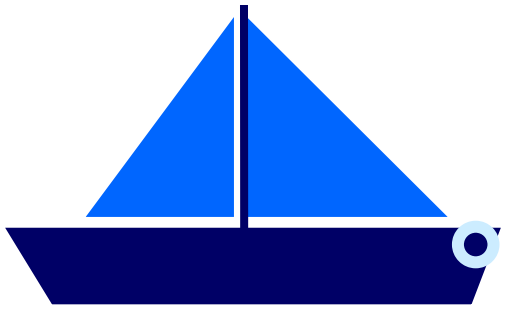
- Opportunities
  - Applicable to all patients
  - Deliver dystrophin gene and repair muscle
- Currently in very early stage clinical development
- Challenges
  - Efficiency very low
  - Damaged muscle gives wrong signals to cells
  - Immunity (only with allogenic transplantation

PTC124/ataluren

1

79





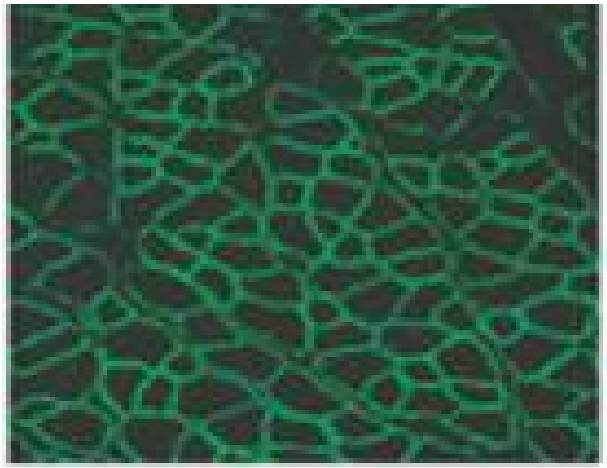
PTC124/ataluren

Cell ignores new stop signal
Complete protein is made

PTC124/Ataluren

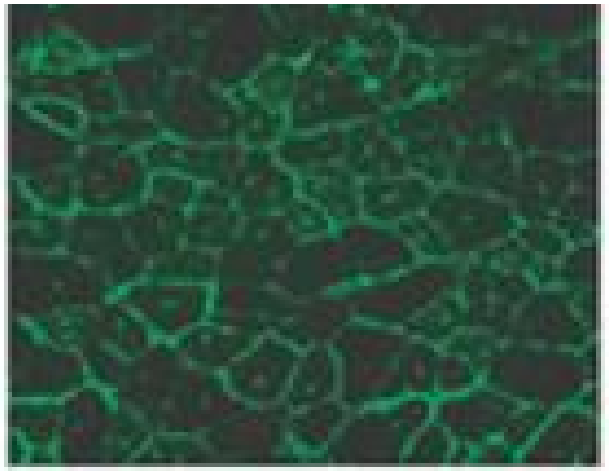
- Tested in patient-derived cells
- Tested in *mdx* mouse model
- Dystrophin restoration

Control



Mdx

Mdx + PTC124



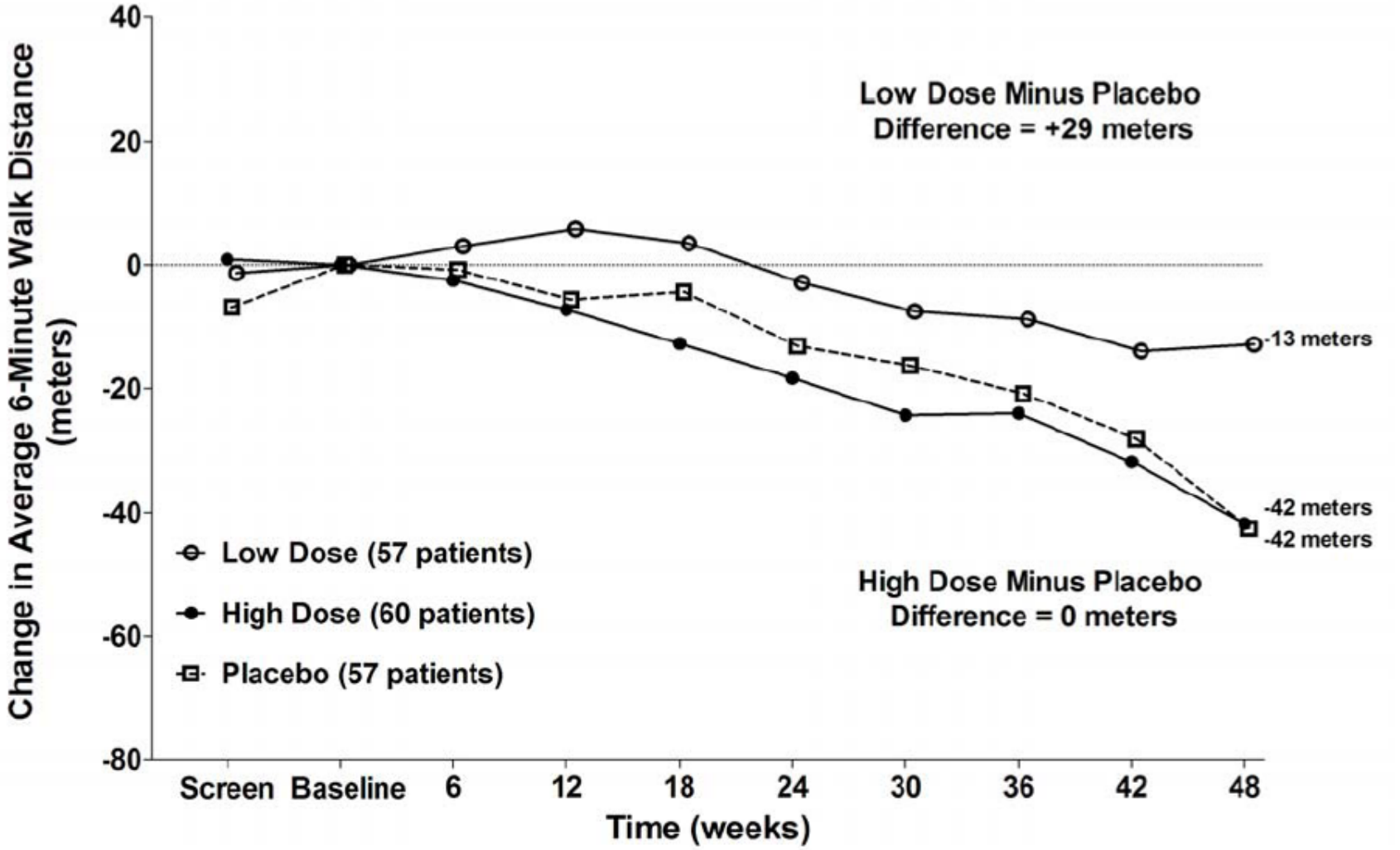
Welch et al, Nature 2007, 447: 87-91

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)

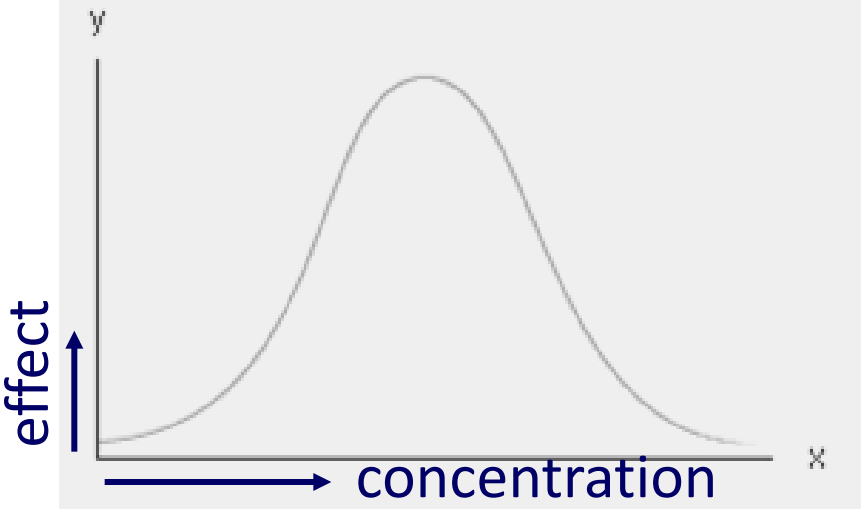
PTC124/Ataluren

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



PTC124/Ataluren future

- Dosing not optimal
  - Bell shaped curve
- Research ongoing
  - Optimize dosing
  - Subset of responders?
- Extension studies re-initiated in USA and Europe
- Planning for new clinical trials



Summary

- Problems Duchenne caused by lack of dystrophin
- Treatments aim to tackle on or more downstream consequences
- Some approaches are mutation specific: need DNA diagnosis
- Lot is known about Duchenne ➔ research paid for by grants from patient organisations
- No therapy yet
- Better care immense impact on quality of live and survival

Thank you!

Parent Project Muscular Dystrophy

Prinses Beatrix Fonds VOOR SPIERZIEKTEN

TREAT-NMD Neuromuscular Network

Duchenne Parent Project

SenterNovem
Agentschap voor duurzaamheid en innovatie

SPIEREN VOOR SPIEREN
www.spierenvoorspieren.nl

ZonMw