Leids Universitair Medisch Centrum

# Therapies in development for Duchenne
# Focus on gene therapy and exon skipping

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# Disclosures

- Employed by Leiden University Medical Center (LUMC), which has patents on exon skipping technology, some of which are licensed to BioMarin and sublicensed to Sarepta. As co-inventor, I am entitled to a share of royalties

- Ad hoc (past) consultant for: AstraZeneca; BioMarin Pharmaceuticals; Dyne; Eisai; Eli Lilly; Galapagos (Alpha Anomeric, Global Guidepoint and GLG consultancy, Grunenthal, Wave and BioClinica); PTC Therapeutics; REGENXBIO; Sarepta Therapeutics; SpliSense; Takeda & Italfarmaco. Remuneration paid to LUMC

- Member of the scientific advisory boards of: Hybridize Therapeutics; Sarepta Therapeutics; Silence Therapeutics & Sapreme. Remuneration paid to LUMC

- LUMC received speaker honoraria from: Alnylam; BioMarin Pharmaceuticals; Pfizer; Italfarmaco, PTC Therapeutics

# Outline of presentation

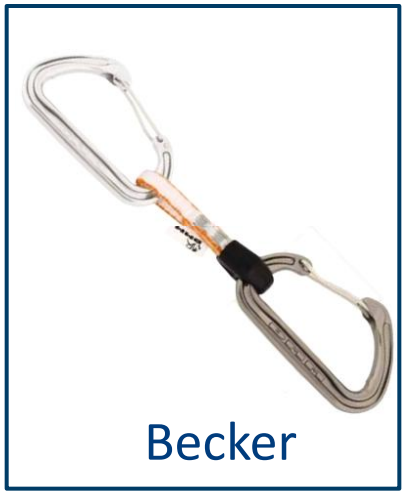
Approaches to restore dystrophin

- Introduction to dystrophin
- Gene therapy
- Exon skipping
- Summary and perspective

# Duchenne: no dystrophin

- Dystrophin stabilizes muscle fiber during contraction
- No dystrophin: continuous damage
- Consequence
  - Chronic inflammation
  - Scar tissue (fibrosis and adipose tissue)
  - Regeneration / repair impaired
  - Loss of muscle tissue and function
- Solution: restore dystrophin



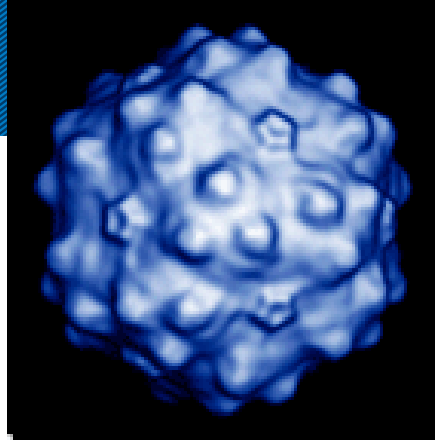


# But first: Challenges for muscle diseases

- Muscle is very abundant
  - 30-40% of our bodyweight is skeletal muscle
- Muscle is not a single organ
  - We have over 700 skeletal muscles
  - Almost all are affected in Duchenne patients
- Muscle replacement by fat/fibrosis is irreversible
  - Function lost cannot be recovered
  - Time of intervention matters

# Gene therapy

# Micro-dystrophin and AAV



- Provide functional gene copy (dystrophin) to muscle
- Delivery to muscle is only efficient with AAV
  - No AAV exposure in the past (immunity)
- Small virus → only small genes fit
  - Components:
    - Viral sequences (so it goes in particles)
    - Promotor (volume switch)
    - Dystrophin (micro-dystrophin) code

# For Duchenne: minimalist approach


Sarepta & Roche (AAV74)/Genethon (AAV8)


Pfizer (AAV9) (For-Mov)


Solid (AAV9)


RegenXBio (AAV8)

# Considerations

- AAV not pathogenic during normal infection
- For Duchenne doses used are very high
- This does cause (severe) side effects
  - Liver has very high exposure
- Immune response will occur
  - Innate & Adaptive
  - This will cause side effects
  - This will prohibit retreatment

# Current state of the art **all approaches**

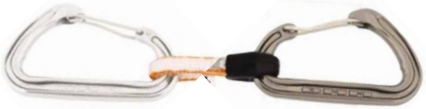
- Clinical studies Solid, Pfizer, Sarepta/Roche, Regenxbio, Genethon
- Only patients without antibodies for  AAV9/7.4/8 included
- General findings >1000 Duchenne patients (trials & commercial)
  - Micro-dystrophin expressed in muscle
  - Side effects
    - Severe to very severe (5 deaths, 3 Pfizer trials, 2 Elevidys)
    - Related to dose (but so are micro-dystrophin levels)
    - Age related (higher dose, worse physical condition)

# Side effects: different types and causes

- Acute
  - Nausea, liver damage, kidney failure, sepsis, death
  - Due to immediate (immune) response to AAV
- Later
  - Muscle breakdown (rhabdomyolysis) and myocarditis
  - Immune response to micro-dystrophin
  - Only seen in patients with large deletion at start of gene
  - How come?

# Immune response to micro-dystrophin

- Immune system reacts to foreign proteins
- Duchenne patients: no dystrophin
- Why doesn’t micro-dystrophin trigger immune response for all?
- Smaller dystrophin isoforms: in >99% of patients
- Start of dystrophin: most patients
- Except those with large deletion in start of gene



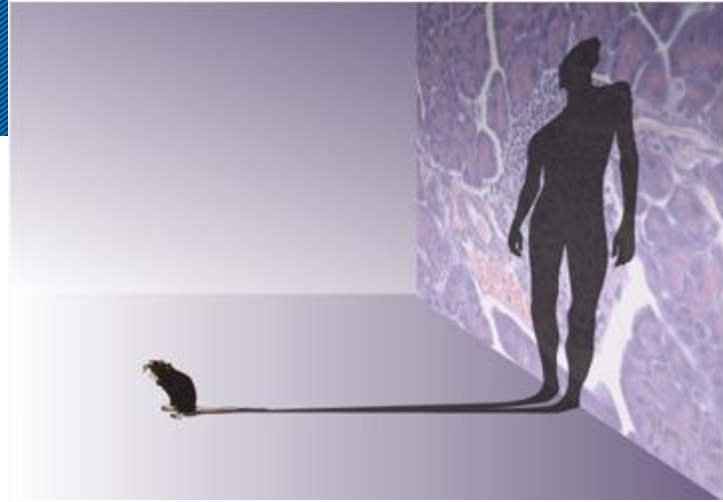
# Functional effects so far for gene therapy

- Clear micro-dystrophin produced in skeletal muscles
- Function seems better than natural history for Elevidys and Pfizer micro-dystrophin (fordadystrogene movaparvovec)
  - But treated patient high dose steroids
- Placebo-controlled data so far
  - Study 1 Elevidys: no difference (but suboptimal dose)
  - Study 2 Elevidys : NSAA primary endpoint not met (48 weeks)
  - Phase 3 study Pfizer did not meet primary endpoint (48 weeks)
  - Phase 3 Elevidys studies Roche/Sarepta ongoing/on hold

# Current state of the art **Elevidys**

- Elevidys approved and over 800 patients treated
  - **CHMP (EMA) did not approve Elevidys (negative opinion, July 2025)**
  - Approved in USA, UAE, Bahrain, Qatar, Kuwait, Omam & Japan
  - Ambulatory patients 4 and over
    - Based on dystrophin restoration
    - Confirmatory trial did not meet primary endpoint
    - Secondary endpoints improved (some significantly)
  - Accelerated approval for non ambulatory patients → confirmatory studies to evaluate functional effects (on hold)
  - Deletions should not involve exon 8-9

# Challenges



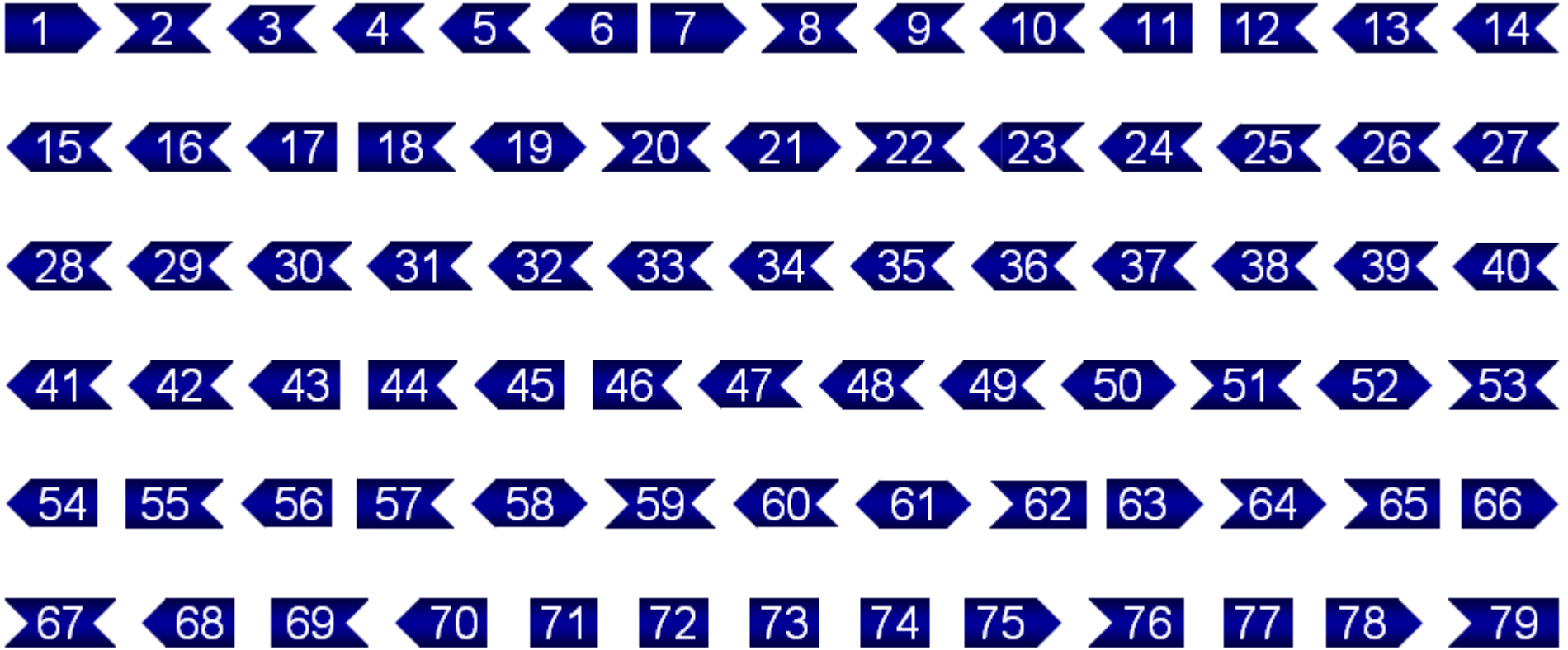
- How functional are micro-dystrophins?
  - Mice/dog vs humans
  - No evidence yet of slower trajectories!
- Longevity of micro-dystrophin expression?
  - Micro-dystrophin levels will go down with time
- Immune response to AAV
  - Cannot treat patients with preexisting antibodies, cannot retreat
- Immune response to micro-dystrophin (selected mutations)
  - Some patients excluded

# Future perspective

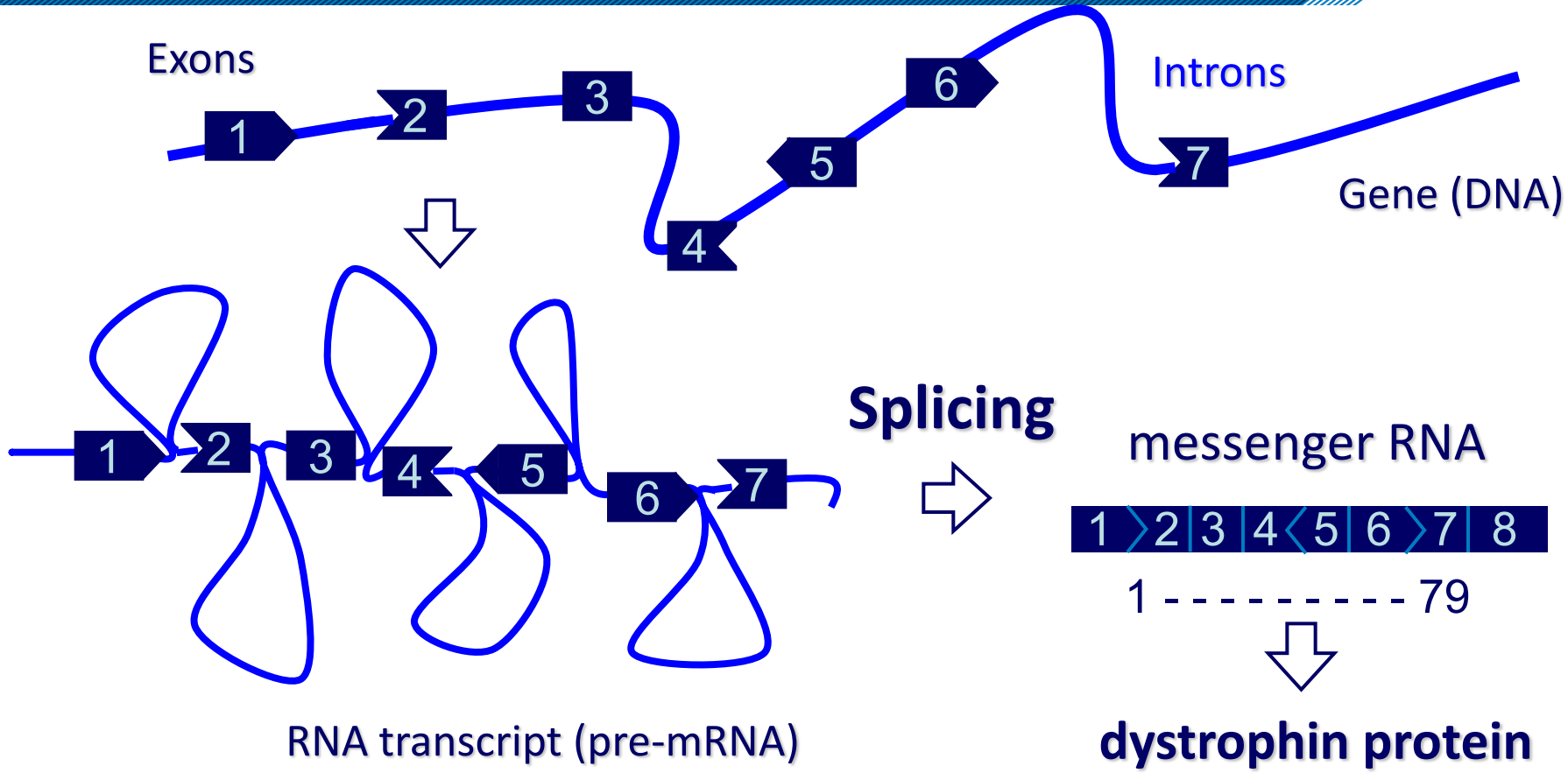
- Need to monitor treated patients
  - Functional effects?
  - Side effects?
- Ways to delivery larger (more functional) dystrophins (intein system)
- Improve AAVs
  - MyoAAV (Solid)
  - Other delivery methods (nanoparticles, early stage)
- Allow treatment of all patients
  - Reduce immunogenicity micro-dystrophin
  - Study how to treat patients with preexisting AAV immunity (ongoing)

# Exon Skipping

# Dystrophin exons



# Dystrophin exons



# Frame shift: Duchenne


Disrupted reading frame

↓

Protein translation truncated prematurely

↓

Dystrophin not functional

# Becker: reading frame maintained


Reading frame not disrupted

↓

Protein translation continues

↓

Dystrophin partly functional

# Exon skipping to restore reading frame


Reading frame restored


Partially functional dystrophin

# What are antisense oligonucleotide drugs?

- Small pieces of modified DNA or RNA
  - Synthesized from chemically modified nucleotides
- Target RNA in a sequence specific manner
- Aim: therapeutic effect
  - Knockdown of toxic protein
  - **Restoration of missing protein**
- Effects are temporary
  - Repeated treatment needed

# Current state of the art

- 4 AONs approved (USA/Japan/Israel)
  - Exon 51, 53 & 45 → 30% of patients
- Based only on dystrophin restoration at low levels (<1-5%)
- Confirmatory studies to assess effect on disease progression ongoing (Viltolarsen results now in)
- Clear: room for improvement
- Bottleneck: delivery to muscle and heart
  applicability

# To consider: dystrophin levels

- Trials mention dystrophin levels
- Most patients make some dystrophin

  So measure increase after treatment vs baseline
- Difficult to compare between companies

  Slight variations in methods, normalizing etc

  Variation in when biopsy taken in trial
- Differences for different exons

  Exon 44 skipping seems easier

# Exon skipping

- How to improve?
  - Chemistry
  - Improving delivery in general
  - Improving delivery to muscle
  - Antisense gene

# Validation and additional exons for PMO

- FDA approved ASO based on dystrophin restoration, weekly IV
- FDA asked for confirmatory studies; also crucial for EMA approval
- Currently evaluated (trials ongoing, fully recruited)
  - Sarepta (eteplirsen (51), golodirsen (53), casimersen (45)
    - MISS51ON (51)
    - ESSENCE (45 & 53)
  - NS Pharma (viltolarsen (53))
    - RACER53X: no difference in trajectory after 1 year
- NS Pharma brogodirsen (44 skipping): 10-15% dystrophin increase
- NS Pharma exon 50 skipping trial ongoing

# Chemical modifications

- Use different chemical modifications
  - Improve affinity of ASO to target → better efficiency
  - Improve circulation time in blood
  - Reduce dosing frequency
- Currently evaluated by
  - BioMarin (BMN351) exon 51 trial recruiting
  - SQY51 (tcDNA) exon 51 trial recruiting
  - Wave (WVE-531) exon 53 trial ongoing

So far results: 5.5% increase dystrophin

# Improving delivery in general

- Use arginine rich peptides
  - Improve delivery to all tissues (also muscle)
  - Therapeutic index: what comes first efficiency or toxicity?
- Currently evaluated by

| | | |
|---|---|---|
| • Sarepta (vesleteplirsen) | exon 51 | development stopped |
| • Pepgen | exon 51 | develoment stopped |
| • Entrada | exon 44 | trial ongoing |
| | exon 45 | trial ongoing |
| | exon 50 | trial planned |

# Improving delivery to muscle and heart

- Use receptor for transferrin (transferrin receptor 1)
  - Improve delivery to muscle and heart
  - Challenge: reaction to antibodies possible, infusion reactions
- Currently evaluated by
  - Avidity (AOC1044)* exon 44 Results in/OLE ongoing

    25% increase in dystrophin
  - Dyne (Dyne-251) exon 51 DELIVER Recruiting

    up to 3.7% increase in dystrophin

*Delpacibart zotadirsen

# Exon skipping via antisense ‘gene’

- Use AAV to deliver antisense gene, more permanent effect
- U7snRNP used, antisense part targets exon 2
- Tested in 3 patients with exon 2 duplication (Kevin Flanigan)
  - <10 dystrophin restoration in patient 1 and 2
  - >80% dystrophin in patient 3 (treated as an infant)
- Challenge
  - Safety concerns AAV (preexisting immunity, side effects)
  - Developing antisense gene for different mutations
  - Currently no ongoing effort to further this

# Exon skipping summary

- Approved exon skipping approaches require weekly intravenous dosing with high doses
- Clinicial trials ongoing to
  - Assess functional effects of approved appraoches
  - Evaluate improved approaches (lower doses, less frequent dosing)
- NB: Becker-type dystrophins produced
- Mutation specific approach: focus on exons where skipping applies to larger cohorts for now

# Summary dystrophin restoring approaches

- Possible to restore dystrophin with exon skipping & AAV-micro-dystrophin, but room for improvement
- Treatments do not restore normal dystrophin (pathology continues)
- Pathology targeting approaches approved (vamorolone and givinostat)
- Future: likely combination therapy

- All **therapies are applied on top of multidisciplinary care!**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