Antisense-mediated exon skipping
Applying lessons learnt from Duchenne muscular dystrophy to other rare diseases

Annemieke Aartsma-Rus
April 16 2013
Becker vs Duchenne
Exon 51 skipping

Reading frame restored

Partially functional dystrophin
AONs

- Modified pieces of RNA/DNA
- Many different modifications possible
- We use 2’-O-methyl phosphorothioate
- Transfection needed in cell culture
- Saline injection possible in vivo
Exon 51 skipping in Δ exon 48-50 cells

NT

48 post transfection

MANDYS1

MANDYS1

DYS2

47 51 52

47 52

MNT 51 -RT

0h 4h 8h 16h 24h 48h HC

NT 48 post transfection
## Applicability

### Cytoskeleton binding domains

<table>
<thead>
<tr>
<th>Exon</th>
<th>All mutations</th>
<th>Deletions</th>
<th>Duplications</th>
<th>Small mutations</th>
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</thead>
<tbody>
<tr>
<td>51</td>
<td>13.0%</td>
<td>19.1%</td>
<td>0.3%</td>
<td>3.0%</td>
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<tr>
<td>45</td>
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<td>11.8%</td>
<td>0.2%</td>
<td>2.2%</td>
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<td>53</td>
<td>7.7%</td>
<td>11.4%</td>
<td>0.1%</td>
<td>1.5%</td>
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<td>44</td>
<td>6.2%</td>
<td>8.85</td>
<td>0.4%</td>
<td>2.7%</td>
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<tr>
<td>46</td>
<td>4.3%</td>
<td>6.2%</td>
<td>0.2%</td>
<td>1.6%</td>
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<tr>
<td>52</td>
<td>4.1%</td>
<td>5.7%</td>
<td>0.5%</td>
<td>2.3%</td>
</tr>
<tr>
<td>50</td>
<td>4.0%</td>
<td>5.6%</td>
<td>0.2%</td>
<td>1.9%</td>
</tr>
</tbody>
</table>

### ECM binding domain

*hotspot*
Intramuscular trial (Prosensa)

0.8 mg in TA

Van Deutkom NEJM 2007; 357: 2677-86
The *mdx* mouse

- Mutation in exon 23 ➔ No dystrophin
- Muscle is dystrophic
- Muscle function impaired
- Needs exon 23 skipping
Systemic studies in *mdx* mouse model

AON levels in muscle and Liver

![Bar chart showing AON levels in different tissues of WT and Mdx mice](chart.png)
Delivery method

AON Tissue levels (ug/g)
Stable Dystrophin was detected in All Treated Samples and Dose Response Observed

*Revertant Fiber

Dystrophin (ManDys 106)
Back to mouse

• Dosing regimens
• 200 mg/kg for 8 weeks SC
  • Daily 28.6 mg/kg
  • Biweekly 100 mg/kg
  • Weekly 200 mg/kg
Exon skipping

- 1x200 mg/kg/wk
- 2x100 mg/kg/wk
- 7x28.6 mg/kg/wk

Exon23 skipping (%)

G Q TA Tri He Di

[Bar chart showing exon skipping percentages for different treatments and genotypes]
How much dystrophin needed?
Survival (long term)

FASEB 2013 van Putten et al
Past challenges

- Limited natural history data
- Limited outcome measures (6MWT)
- No biomarkers
- Preclinical studies not standardized
- Rare disease ➔ multiple trials sites needed
  - Care standards
  - Trial sites where?
- Personalized, mutation specific approach
  - Registries needed
What is TREAT-NMD (a recap!)

- A “network of excellence” initially funded by the European Union (but with global collaborations)
- Aims to help promising new treatments for neuromuscular diseases make the transition from the lab to the patient
- Not a research project but an infrastructure project
- Creating the “tools” for trial-readiness in the neuromuscular field
- Helping researchers and expert centres collaborate better
- Improving patient care worldwide

➤ Sustained beyond 2011 as TREAT-NMD Alliance
TREAT-NMD new developments

• 2007-2011: Network funded by the European Union

• 2012 onwards: TREAT-NMD Alliance funded through multiple funding streams and with global partners
  - Led by an elected Executive Committee
  - Supported by academic advisory board (“task force”) of NMD leaders
  - 3-year “action plan” of key areas where global collaboration is required
  - Newly funded research projects (NEUROMICS!)
  - New Alliance charter and membership
  - New focus on additional neuromuscular conditions
TREAT-NMD Alliance Executive Committee

- 12 members, global representation (Europe, USA, Australia, Japan)
- Clinicians, researchers and patient representatives
- Chair: Hanns Lochmüller, UK; vice-chair: Annemieke Aartsma-Rus, NL
- Monthly teleconferences (up to 2 hours long!)
- New Alliance charter (September 2012)
Three year work plan

- **Joint Research for DMD**
- **Standard Operating Procedures**
- **BioBank**
- **Patient Registries**
- **Care & Trial Site Registry**
- **Outcome Measures**
- **Standards of Diagnosis & Care**
- **TACT**
- **Website & Communications**

**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
## Splice modulation for RDs

*(not exhaustive)*

<table>
<thead>
<tr>
<th>Disease</th>
<th>Stage</th>
<th>Mechanism</th>
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<tbody>
<tr>
<td>Duchenne muscular dystrophy</td>
<td>Phase II &amp; III trials</td>
<td>Reading frame restoration</td>
</tr>
<tr>
<td>Spinal muscular atrophy</td>
<td>Phase I trial</td>
<td>Exon inclusion</td>
</tr>
<tr>
<td>β-thalassemia</td>
<td>Mouse model</td>
<td>Blocking cryptic splicing</td>
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<tr>
<td>Hutchinson-Gilford progeria</td>
<td>Mouse model</td>
<td>Isoform switching</td>
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<td>Fukuyama congenital muscular dystrophy</td>
<td>Mouse model</td>
<td>Skip retrotransposon</td>
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<td>Menkes disease</td>
<td>Zebrafish model</td>
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<td>Atherosclerosis</td>
<td>Mouse model</td>
<td>Reading frame disruption</td>
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<tr>
<td>Limb-Girdle muscular dystrophy 2B</td>
<td>Mouse model</td>
<td>Reading frame restoration</td>
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<tr>
<td><strong>Muscle wasting diseases</strong></td>
<td>Mouse model</td>
<td>Reading frame disruption</td>
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<tr>
<td>Cancer</td>
<td>Mouse model</td>
<td>Isoform switching</td>
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<td><strong>CADASIL</strong></td>
<td>Cell cultures</td>
<td>Cysteine quantity correction</td>
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<td>Huntington Disease</td>
<td>Cell cultures</td>
<td>Protein modification</td>
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<tr>
<td>Spinalcerebellar ataxias</td>
<td>Cell cultures</td>
<td>Protein modification</td>
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<tr>
<td>Leber congenital amourosis</td>
<td>Cell cultures</td>
<td>Blocking cryptic splicing</td>
</tr>
<tr>
<td>Methalmalonic/Propionic acidemia</td>
<td>Cell cultures</td>
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</tbody>
</table>
Current challenges AON development

- Personalized medicine approaches
  - (Subsets of) rare diseases
  - Limited patient population
- Regulatory models unfit for RDs
- Harmonization of research needed
  - Within an RD
  - Between RDs
- Jointly overcome hurdles and face challenges
Networking towards clinical application of antisense-mediated exon skipping in rare diseases

Annemieke Aartsma-Rus
Leiden University Medical Center, the Netherlands

COST Domain - Biomedicine and Molecular Biosciences (BMBS)
Objectives of the network

Networking

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

To

- Pave the way for exon skipping therapy development for rare diseases
- Exploit exon skipping for as many patients as possible
- Foster synergistic work and jointly tackle challenges
- Anyone who can contribute within selected countries can link up! (mail me: a.m.rus@lumc.nl)
Acknowledgements

**Human Genetics**
Annemieke Aartsma-Rus
Linda Switzar
Christa Tanganyika-de Winter
Laura van Vliet
Nisha Verwey
Maaike van Putten
Margriet Hulsker
Ingrid Verhaart
Willem Hoogaars
Marcel Veltrop
Isabella Gazzoli
Pietro Spitali
Silvana Jirka
Monika Hiller
Gert-Jan van Ommen

**LUMC – Neurology/Radiology**
Jan Verschuuren
Beatrijs Wokke
Janneke van den Bergen
Hermien Kan
Louise van der Weerd

**Prosenza BV**
Judith van Deutekom
Anneke Janson
Giles Campion
Sjef de Kimpe
Jessica Sipkens