Exon Skipping: Duchenne Muscular Dystrophy

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Duchenne dystrophy =
Absence of dystrophin
Complete loss of function

Becker dystrophy =
Present, but abnormal
Partial loss of function

Large in-frame deletions
Can be clinically very mild, asymptomatic (hyperCKemia)
How Exon-Skipping Works
Mad Dog Can Run But Not Eat

Full length dystrophin
Normal muscle

Nonsense mutation
No dystrophin

Forced skipping of 2 additional exons

Semi-functional dystrophin
Clinical applications of anti-sense:

- 20 yrs; 90 clinical trials; 40 completed
- >2,000 patients, targeting cancer, inflammatory disease, and other indications.
- A single AO has been FDA approved
  - Vitravene®, intraocular injection to inhibit cytomegalovirus retinitis (CMV) in immunocompromised patients; Isis Pharmaceuticals
  - No longer marketed.

Anti-sense barriers
- Sufficient intracellular drug for biochemical efficacy
- Toxicity
Systemic anti-sense in Duchenne

- ~100-fold better than other disorders
  - Drug entry to cells facilitated by overt breaches in myofiber cell membranes (bulk flow into cell)-10 fold
  - Rescue (dystrophin) vs. known down (others) – 10 fold
    - Previous knock-downs: goal 90% of mRNA targets
    - Dystrophin mRNA rescue: 10% of mRNA targets

Prosensa/GSK

AVI Biopharma
Proof of principle: Large animal dog model

• Spontaneous mutations in dogs
• Similar to human disease
  • Progressive weakness, death by 6 months
• Challenging mutation
  • Splicing near amino-terminus;
  • Required targeted 2 exons
• Tested drug combinations by intramuscular injection

Non-treated littermate  3 morpholino treated CXMD
Systemic Delivery

Endpoints:
1. Dystrophin by blot, Immunostaining
2. Histology, Functional testing, Symptom grading, MRI
3. Toxicology

Yokota et al. Annals Neurology 2009
Recovery of dystrophin expression after systemic morpholino treatment in CXMD

Cocktail morpholinos (5 inj x 120 mg/Kg in total) treated CXMD

Dystrophin (Dys-1) and nuclear staining at 15 days after 5 x injections with 6 g of morpholinos in total targeting exon 6 and 8 (cocktail of Ex6A, Ex6B, Ex8A) into young adult CXMD. Bars: 100 µm
Dogs and Morpholinos: Dystrophin rescue: Variable, best ~20%

Wild-type Tibialis Anterior (1/2 Dilution)  Wild-type (1/10 Dilution)  CXMD non-treated (TA)

Triceps Brachii  Biceps Brachii  Diaphragm  Esophagus  Tibialis Anterior  Adductor magnus  Extensor digitorum longus  Masseter  Heart

5 x 120 mg/Kg Morpholino treated

DYS1 (dystrophin)

Desmin

50 µg, 10 µg, or 100 µg of total proteins were loaded in each lane as indicated.
15 m Running test before and after morpholino injection

- Pre-injection (5 months of age)
- After 5 x systemic injection of morpholinos (7 months of age)

-morpholino injected dog

non-injected littermate 1

non-injected littermate 2
Morpholinos: GLP Toxicology

- Normal animals/humans
  - Will drugs skip normal gene, induce DMD?
- EMEA: Less stringent requirements for Tox
  - Do tox in DMD patients
- FDA – more tox studies required (AVI; DoD; FED; CureDuchenne)
  - Human DMD drug (AVI 4658)
    - Normal mice – 12 wk weekly IV dose: 960 mg/kg/wk
    - Non-human primates – 12 wk weekly IV dose: 320 mg/kg
  - Mouse mdx drug (AVI 4225)
    - Mdx mice – 12 wk weekly dosing up to 960 mg/kg

- Consistent, predictable pharmacokinetics
- Subcutaneous less effective than IV
- Traditional dose equivalencies: permits dosing humans to 100 mg/kg
Status of clinical development programs: completed studies

- Morpholinos (AVI)
  - Open label dose escalation study
    - Francesco Muntoni, UCL
    - 19 patients, 0.5 – 20 mg/kg/wk IV, 12 wks
    - Convincing dystrophin expression in muscle; few patients at potentially therapeutic levels

- 2’ Omethyl (Prosensa/GSK)
  - Open label dose escalation study
    - Nathalie Goemens, Leuven
    - 12 patients, 0.5 – 6 mg/kg/wk subcutaneous, 5 wks
    - + 12 wk extension at 6 mg/kg/wk
    - Some dystrophin expression
Ongoing 2’Omethyl Clinical Studies

- **Study DMD114117** (regime optimization, EU, Australia, Turkey, Israel)
  - Ambulant, double blind placebo-controlled, two dosing regimes vs placebo, 12 sites

- **Study DMD114118** (single dose PK, USA + France)
  - Non-ambulant, single dose, dose-escalating tolerability and PK, 2 sites

- **Study DMD114044** (pivotal, global excl. USA)
  - Double-blind, placebo-controlled, 6mg/kg vs placebo, 35 sites

- **DMD114876 (USA)**
  - 2 different doses of SC GSK2402968 versus placebo administered over 24 weeks in ambulant subjects with DMD.

Ongoing Morpholino Clinical Studies

- **Two dose, blinded, placebo-controlled (dose-finding, USA- AVI)**
  - 30 mg/kg; 50 mg/kg wk IV; 12 patients; 12 wk with extension study
Completed Phase I/IIa open label dose escalation study + 12 week extension reported

**Endpoints**
- Safety and tolerability
- Plasma and tissue pharmacokinetics
- Muscle biopsies: RNA and protein effects
- Muscle strength and function

**Safety and efficacy assessments**
- Weekly: AEs, urinalysis (Weeks 1–16)
- 2–weekly: thrombocytes, urinalysis (Weeks 16–96)
- Monthly: safety, blood and urine, PK (to Week 24), ECGs, muscle strength and function (Weeks 8–96)
Completed Phase I/IIa open label dose escalation study + 12 week extension reported

**Summary:**
- First successful systemic administration of GSK2402968
- Favorable pharmacokinetic profile
- Dose dependent increase in dystrophin expression
- Well tolerated at 6 mg/kg sc (12w extension)

**Selected dose:** 6 mg/kg

Tissue levels of GSK2402968 in muscle biopsy at this dose: 6.9 ± 1.9 µg/g
Summary

- GSK2402968 was generally well tolerated after 96 weeks
  - Renal effects, thrombocytes and local injection-site reactions warrant continued monitoring
    - Reversibility of renal effects during off-treatment period was observed after intermittent dosing
- Considering the expected disease progression, encouraging results in 6-minute walk distance were observed in 7 out of 10 ambulant boys (P4.27)
  - Larger placebo-controlled studies (DMD114117 and DMD114044) are currently ongoing
Exon skipping and dystrophin restoration in patients with Duchenne muscular dystrophy after systemic phosphorodiamidate morpholino oligomer treatment: an open-label, phase 2, dose-escalation study


Summary

Background We report clinical safety and biochemical efficacy from a dose-ranging study of intravenously administered AVI-4658 phosphorodiamidate morpholino oligomer (PMO) in patients with Duchenne muscular dystrophy.

Method We undertook an open-label, phase 2, dose-escalation study (0.5, 1.0, 2.0, 4.0, 10.0, and 20.0 mg/kg bodyweight) in ambulant patients with Duchenne muscular dystrophy aged 5–15 years with amenable deletions in DMD. Participants had a muscle biopsy before starting treatment and after 12 weekly intravenous infusions of AVI-4658. The primary study objective was to assess safety and tolerability of AVI-4658. The secondary objectives were pharmacokinetic properties and the ability of AVI-4658 to induce exon 51 skipping and dystrophin restoration by RT-PCR, immunohistochemistry, and immunoblotting. The study is registered, number NCT00844597.

Findings 19 patients took part in the study. AVI-4658 was well tolerated with no drug-related serious adverse events. AVI-4658 induced exon 51 skipping in all cohorts and new dystrophin protein expression in a significant dose-dependent (p=0.0203), but variable, manner in boys from cohort 3 (dose 2 mg/kg) onwards. Seven patients responded to treatment, in whom mean dystrophin fluorescence intensity increased from 8.9% (95% CI 7.1–10.6) to 16.4% (10.8–22.0) of normal control after treatment (p=0.0287). The three patients with the greatest responses to treatment had 21%, 15%, and 55% dystrophin-positive fibres after treatment and these findings were confirmed with western blot, which showed an increase after treatment of protein levels from 2% to 18%, from 0.9% to 17%, and from 0% to 7.7% of normal muscle, respectively. The dystrophin-associated proteins α-sarcoglycan and neuronal nitric oxide synthase were also restored at the sarcolemmal. Analysis of the inflammatory infiltrate indicated a reduction of cytotoxic T cells in the post-treatment muscle biopsies in the two high-dose cohorts.

Interpretation The safety and biochemical efficacy that we present show the potential of AVI-4658 to become a disease-modifying drug for Duchenne muscular dystrophy.
Dystrophin protein expression in patients who responded to treatment
## Comparison of exon 51-skipping therapies using systemic antisense oligonucleotides

<table>
<thead>
<tr>
<th></th>
<th>Goemans and colleagues&lt;sup&gt;5&lt;/sup&gt;</th>
<th>Cirak and colleagues&lt;sup&gt;6&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Compound name</strong></td>
<td>PRO051</td>
<td>AVI-4658</td>
</tr>
<tr>
<td><strong>Chemistry</strong></td>
<td>2′OMe</td>
<td>PM0</td>
</tr>
<tr>
<td><strong>Administration method</strong></td>
<td>Weekly subcutaneous injection</td>
<td>Weekly intravenous injection</td>
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<tr>
<td><strong>Dose (mg/kg body weight)</strong></td>
<td>0.5, 2, 4, and 6</td>
<td>0.5, 1, 2, 4, 10, and 20</td>
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<tr>
<td><strong>Frequency of administration</strong></td>
<td>5 weeks and extended 12 weeks</td>
<td>12 weeks</td>
</tr>
<tr>
<td><strong>Total number of patients</strong></td>
<td>12</td>
<td>19</td>
</tr>
<tr>
<td><strong>Deletion exon(s) in patients</strong></td>
<td>45–50, 48–50, 52</td>
<td>45–50, 47–50, 48–50, 49–50, 52</td>
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<tr>
<td><strong>Maximum proportion of dystrophin-positive fibres</strong></td>
<td>100% post-treatment</td>
<td>5% pretreatment, 55% post-treatment</td>
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<tr>
<td><strong>Maximum dystrophin signal intensity to control muscles by immunofluorescence</strong></td>
<td>15.6% post-treatment</td>
<td>11% pretreatment, 27% post-treatment</td>
</tr>
<tr>
<td><strong>Maximum dystrophin protein level to control muscles by western blotting</strong></td>
<td>Not available</td>
<td>5% pretreatment, 18% post-treatment</td>
</tr>
<tr>
<td><strong>Serious adverse events</strong></td>
<td>None</td>
<td>None</td>
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</tbody>
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Cirak and colleagues<sup>6</sup> calculated the success rate after subtracting the endogenous background level for each patient using a pretreatment biopsy, whereas Goemans and colleagues did not.<sup>5</sup>

*Table: Comparison of exon 51-skipping therapies using systemic antisense oligonucleotides*
AVI BioPharma Press Release: Phase II b trial

• 50 mg/kg/IV/wk showed little evidence of dystrophin expression after 12 wks

• 30 mg/kg/IV/wk for 24 wks showed reasonable dystrophin (about 20% of myofibers by IF)

• No one showed improvements in 6 minute walk

• 6 months of treatment before seeing any dystrophin and no clinical efficacy !!!

  • Is exon 51 a right choice for initial exon skipping studies?
  • 20% of myofibers by IF may still be less than 10% dystrophin by blot, so may not be getting enough dystrophin to show efficacy
  • Study includes older patients, so it may be harder to get dystrophin expression with less muscle left
  • The results are simply variable between patients
  • A new publication has shown that 20% of deletions have complex breakpoints, many with local inversions so some exons that are remaining in a DMD patient may be inverted, leading to unpredictable exon skipping
Challenges and unknowns

- Many exonic targets.
- BMD-like dystrophin function.
- Pre-clinical efficacy studies.
- AO target sequence selection.
- Long-term chronic tox.

- Goal:
  - Multiple exons – reduced regulatory hurdles
  - Coordination of international research community
NIAMS P50: Center of Research Translation on Exon Skipping (Hoffman, Clemens)

Projects

- **Project 1:** Dystrophin mRNA fidelity and protein function.
- **Project 2:** Optimization of AO drugs to exons 45, 51, 53.
- **Project 3:** Becker muscular dystrophy natural history.

Cores

- **Core A:** Administrative.
- **Core B:** In vitro and in vivo functional assays.
- **Core C:** Molecular diagnostics and tissue banking.

Synergistic programs

- U of Pitt CTSA; CNMC CTSA
- CINRG network
- NIH U54 ex45
- DoD Program Project
- National Center for Medical Rehabilitation Medicine
NICHD U54: Pediatric pharmacology center at Children’s National Medical Center

Pediatric toxicity and efficacy in long-term systemic treatment with anti-sense: A case study of personalized medicine.

John Van den Anker, Edward Connor

NICHD Steering Committee

Project 1. Clinical evaluation of urine biomarkers for morpholino accumulation and resolution in renal epithelial cells.

John Van den Anker, Edward Connor, Jerry Mendell

Project 2. Biomarker discovery for AO accumulation in kidney.

Eric Hoffman, Yetrib Hathout

Project 3. Preclinical dosing optimization: Dosing schedule, tissue bioavailability, and functional outcome measures.

Kanneboyina Nagaraju, Qij Lu

Core B. Bioanalytical Core

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