Duchenne muscular dystrophy
Duchenne muscular dystrophy: Clinical features

- X linked recessive – mostly males
- High serum creatine kinase from birth – neonatal screening possible
- Presentation early school years – difficulty keeping up with peers
- Progressive muscle weakness and loss
- Loss of ambulation ~10 yrs, loss of activities daily living 15 yrs - ventilation
- Daily glucocorticoids – increase in strength, delay in loss of ambulation
- Cardiac involvement - failure
Normal Dystrophin

Duchenne muscular dystrophy
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)
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
- Therapeutic window
Systemic anti-sense in Duchenne

- ~100-fold increase in target efficacy
  - Drug entry to cells facilitated by overt breaches in myofiber cell membranes (bulk flow into cell)
  - 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 LOF 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

40-60 mg/kg@AO x 3 AOs
Weekly IV dose = 120-200 mg/kg
Cumulative dose = 1.4 grams/dog
2-4 months treatment
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, average ~20%

<table>
<thead>
<tr>
<th>Wild-type Tibialis Anterior (1/2 Dilution)</th>
<th>Wild-type (1/10 Dilution)</th>
<th>CXMD non-treated (TA)</th>
<th>Triceps Brachii</th>
<th>Biceps Brachii</th>
<th>Diaphragm</th>
<th>Esophagus</th>
<th>Tibialis Anterior</th>
<th>Adductor magnus</th>
<th>Extensor digitorum longus</th>
<th>Masseter</th>
<th>Heart</th>
</tr>
</thead>
</table>

**5 x 120 mg/Kg Morpholino treated**

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)

non-injected littermate 2
non-injected littermate 1
morpholino injected dog
Running test of littermates

Non-treated littermate

11 x weekly treated littermate
GLP Tox

- Normal animals/humans
  - Will drugs skip normal gene, induce DMD?

- EMEA: 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**
AVI-4658 and AVI-4225 Mouse Study Summary

- No dose related changes in urine or serum kidney parameters
- No drug related clinical chemistry findings in all groups
- No test article-related effects on urinalysis parameters
- No adverse clinical observations

**AVI-4225 in mdx mice**: Microscopic evaluation shows improvement in myofiber degeneration as a result of treatment with AVI-4225 in mdx mice

<table>
<thead>
<tr>
<th>Dose level: mg/kg (mdx strain)</th>
<th>0 (IV)</th>
<th>12 (IV)</th>
<th>120 (IV)</th>
<th>960 (IV)</th>
<th>960 (SC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
</tr>
<tr>
<td>Biceps femoris</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-minimal</td>
<td>2 0</td>
<td>5 4</td>
<td>4 4</td>
<td>5 5</td>
<td>5 3</td>
</tr>
<tr>
<td>-mild</td>
<td>5 6</td>
<td>1 5</td>
<td>0 4</td>
<td>0 0</td>
<td>2 4</td>
</tr>
<tr>
<td>-moderate</td>
<td>3 4</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
</tbody>
</table>

IV More effective
SubQ Less effective
GLP Tox: Morpholinos

- Excellent therapeutic window
  - Efficacy ~40 mg/kg
  - GLP Primates 320 mg/kg; Mice 960 mg/kg
    - Morpholinos not metabolized
    - Traditional dose equivalencies: permits dosing humans to 100 mg/kg
- Do not induce DMD in normal muscle
- Consistent, predictable pharmacokinetics
- Subcutaneous less effective than IV
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)**
  - 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: $6.9 \pm 1.9 \mu g/g$

Dystrophin (ManDys 106)
6-Minute Walk Test:
93-Week Extension at 6 mg/kg/week

N=10 (subjects who completed all 6-minute walk test [6MWT] assessments). Subject 103 stopped test early and is not included in Figure; subject 201 was non-ambulant at baseline. Subjects 106 and 107 not able to attempt 6MWT at 93 weeks - still included in mean change.
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
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)

- **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

External Advisory 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, Qi Lu

Core B. Bioanalytical Core

Pedro Jose, Robin A. Felder, Kristy Brown, Patricio Soares da Silva
Acknowledgements

Masanori Kobayashi, Sachiko Ohshima, Yoshitsugu Aoki, Takashi Saito, Kazue Kinoshita, Michiko Wada, Yumiko Yahata, Shin-ichi Ichikawa, Hideki Kita, Satoru Masuda, Takashi Okada, Akinori Nakamura, and Shin’ichi Takeda

Department of Molecular Therapy, and General Animal Research Facility, National Center of Neurology and Psychiatry (NCNP), Kodaira, Tokyo, Japan

Qi-long Lu

Muscular Dystrophy Laboratory, Neuromuscular/ALS Center, Carolinas Medical Center, Charlotte NC, USA

Katsutoshi Yuasa

Faculty of Pharmacy, Musashino University, Tokyo, Japan

Toshifumi Yokota,

William Duddy, Terence Partridge and Eric Hoffman

Center for Genetic Medicine Research, Children’s National Medical Center, Washington DC, USA

Naoko Yugeta

School of Veterinary Medicine, Azabu University, Sagamihara, Japan

Funding: Department of Defense, Foundation to Eradicate Duchenne (FED), Crystal Ball – MDA (Richmond), NIH Wellstone network, Ministry of Health - Japan