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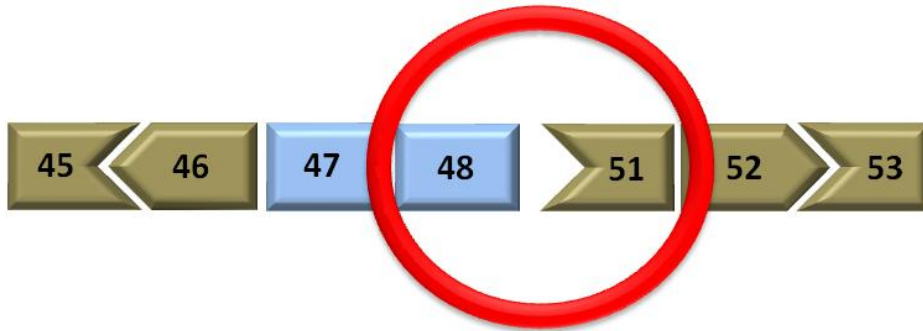
RARE DISEASE WORKSHOP SERIES
Improving the Clinical Development Process

Immunohistochemistry of dystrophin as a surrogate marker in DMD

Diane Frank, PhD
Director, Discovery Research Biology
AVI BioPharma



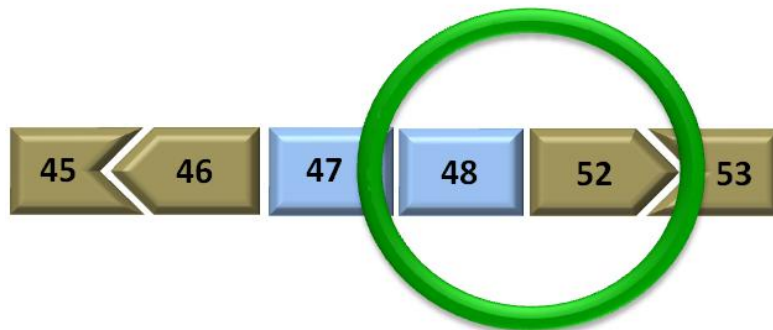
AVI's exon skipping approach enables dystrophin translation



Deletion of exons 49-50 results in an out of frame deletion in mRNA



In this example, AVI's therapeutic compound, eteplirsen, skips exon 51



Successful skip allows for subsequent transcription of in-frame mRNA



Serum biomarkers for DMD

- Serum biomarkers of disease progression allow for monitoring efficacy during treatment periods of early phase clinical trials.
 - Current serum biomarker, creatine kinase, has many major drawbacks
- miRNAs: Muscle specific miRNAs associated with muscle differentiation and regeneration are elevated in plasma from DMD animal models and patients.
 - Serum levels of miR-1, miR-206 and miR-133 are elevated in DMD, BMD, mdx mouse serum and CXMD dog serum.
 - Levels of muscle specific miR-1, miR-206 and miR-133 correlate with DMD progressive state.
- MMP-9, TIMP-1: Serum levels of matrix metalloproteinase-9 and its modulator, Tissue Inhibitor of Metalloproteinase are elevated in DMD.
 - Biomarkers associated with inflammation and matrix remodeling
 - Serum levels correlate with progression of disease, even in non-ambulatory patients where there is substantial muscle degeneration



Criteria for standardized method of quantifying dystrophin protein in muscle biopsies

- Correlate restored dystrophin expression to clinical outcome
- Standardized methods for analysis of both % positive fibers and dystrophin intensity localized to sarcolemma
- Assay is specific for functional dystrophin
 - Measure dystrophin localization and recruitment of associated protein complex to the sarcolemma membrane
- Assay is sensitive and reproducible to allow relative quantitation of low levels of restored dystrophin
 - Protocol optimized to distinguish pre-treatment revertant fibers and residual dystrophin from treatment-induced restored expression



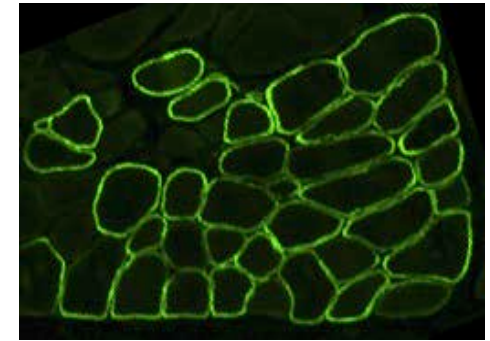
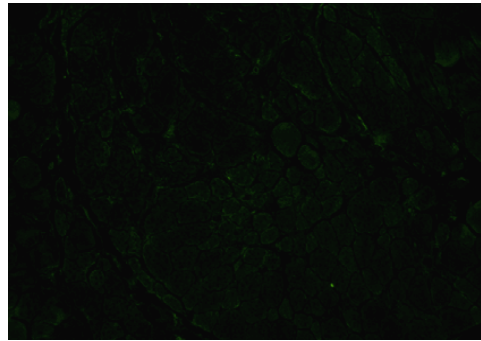
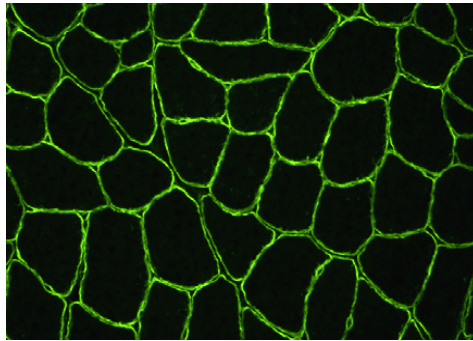
Increase in Dystrophin Detected by Immunofluorescence

NORMAL SUBJECT

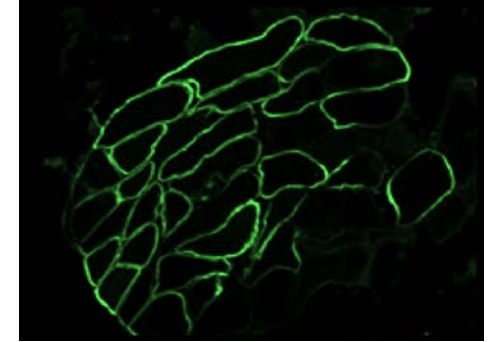
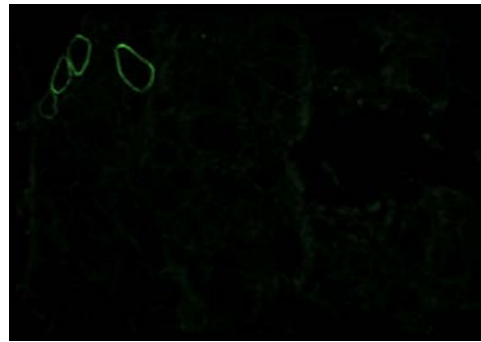
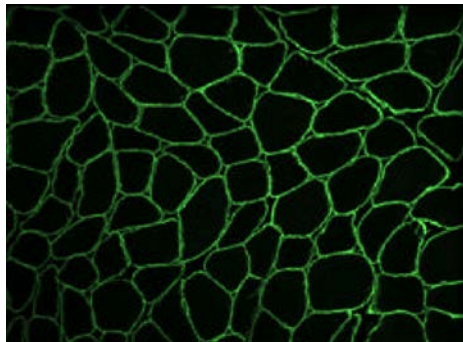
PRE-TREATMENT

POST-TREATMENT

PATIENT 205 (20mg/kg)



PATIENT 201 (2mg/kg)



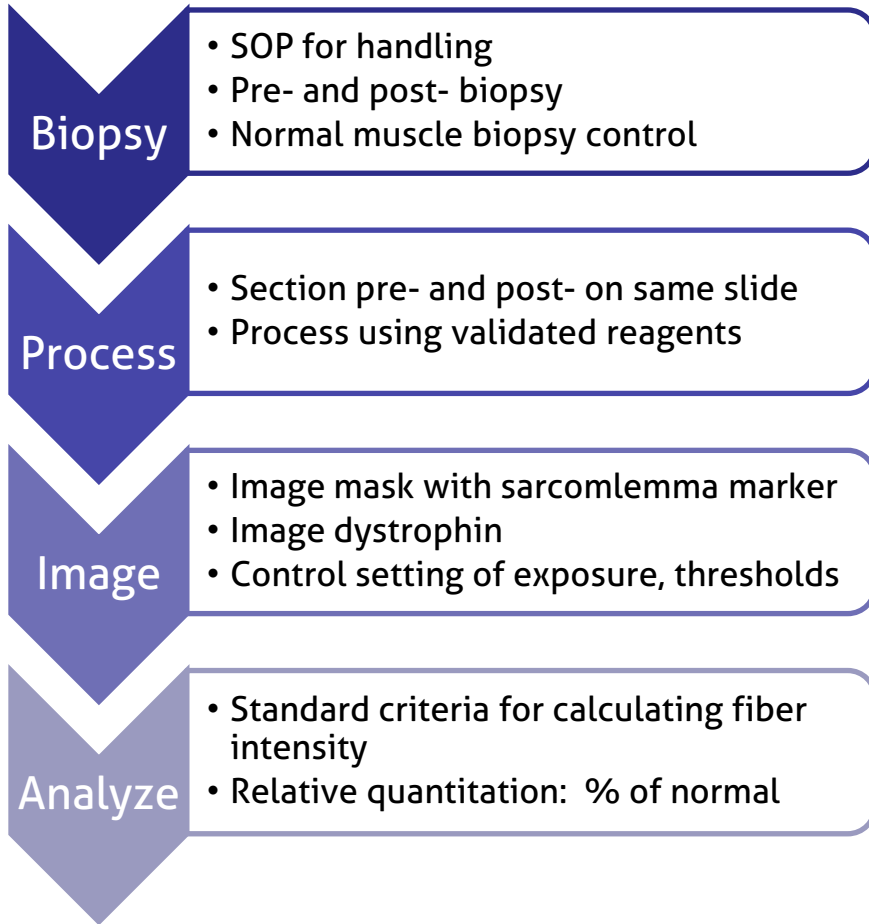


Problems encountered, Current Solutions

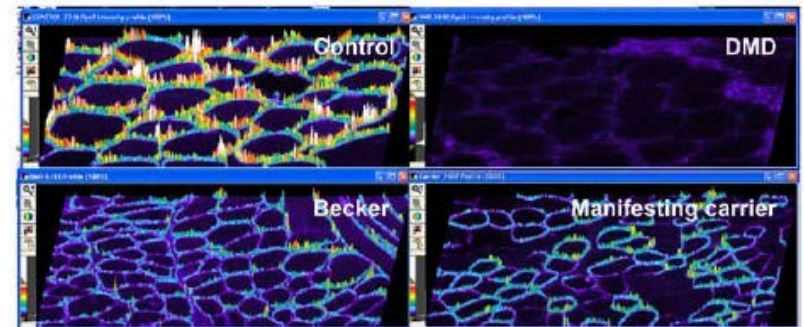
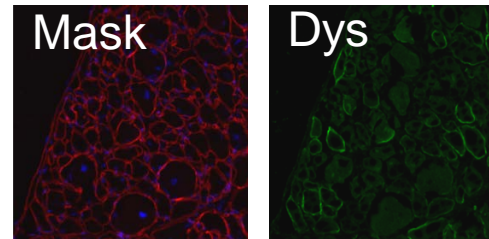
- Imaging technology has not been available in clinical laboratories
 - Currently, automated image acquisition and quantitation is more readily available in clinical pathology laboratories
- Relative quantitation has not been standardized between sites and studies
 - Measurements need to be standardized to normal tissue (“USP reference standard”) to allow inter-site and study comparisons
- Correction for pre-treatment dystrophin levels has not been controlled
 - Image acquisition thresholds need to be consistently set to account for revertant fibers and pre-treatment baseline fiber intensity
- Analysis has been limited to selected areas of biopsy
 - Automated image analysis can more efficiently sample greater area of biopsy



Quantitative Immunohistochemistry Procedure



Digital Pathology system



Arechavala-Gomez, et al. 2009 NAN



Eteplirsen Phase 1b/2 Study: The Lancet July 2011

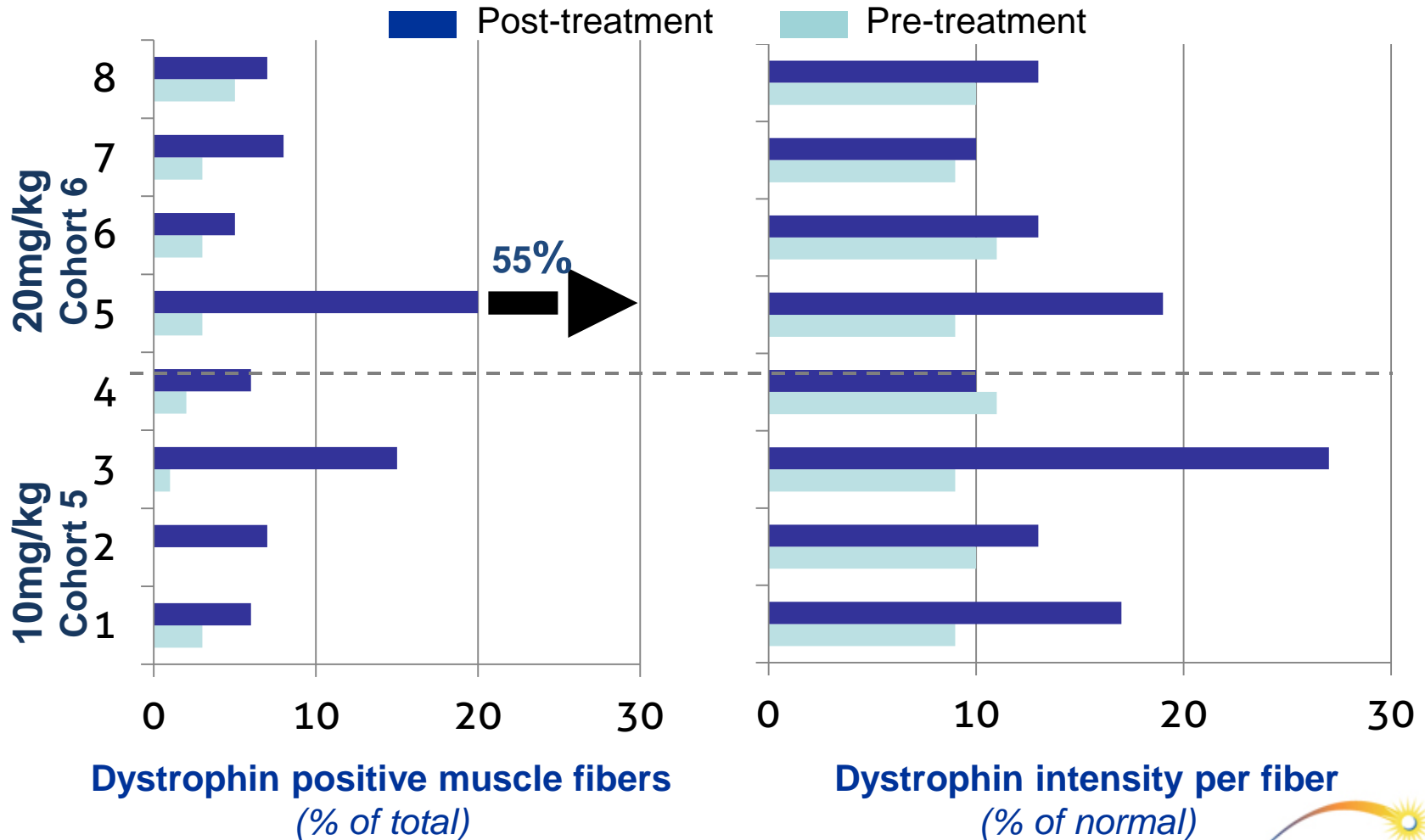
- **Design:** *Open label, multiple dose, dose-ranging study*
 - Weekly dosing for 12 weeks, 14 week post dosing follow-up period
 - Pre and post treatment biopsies
 - Doses: 0.5, 1, 2, 4, 10 and 20 mg/kg
- **Study Population**
 - 19 Ambulatory DMD patients ≥ 5 and ≤ 15 years of age
 - DMD mutation amenable to treatment via exon 51 skip
- **Study Objectives**
 - Evaluate safety and tolerability
 - Measure dystrophin expression restoration via immunofluorescence and western blot
 - Exploratory evaluation of clinical outcome measures

“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”

Sebahattin Cirak, Virginia Arechavala-Gomez, Michela Guglieri, Lucy Feng, Silvia Torelli, Karen Anthony, Stephen Abbs, Maria Elena Garralda, John Bourke, Dominic J Wells, George Dickson, Matthew J A Wood, Steve D Wilton, Volker Straub, Ryszard Kole, Stephen B Shrewsbury, Caroline Sewry, Jennifer E Morgan, Kate Bushby, Francesco Muntoni



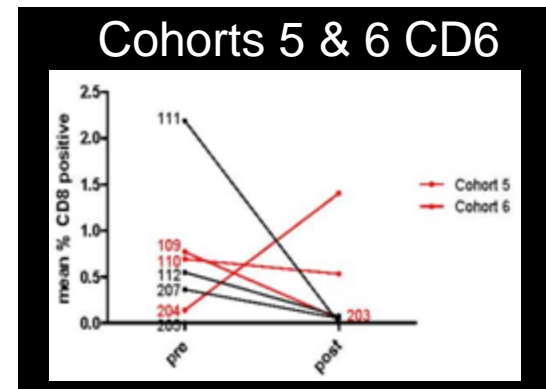
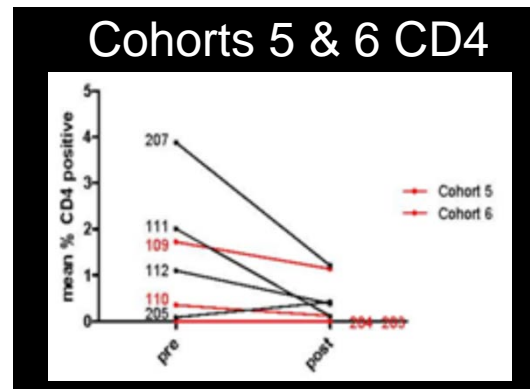
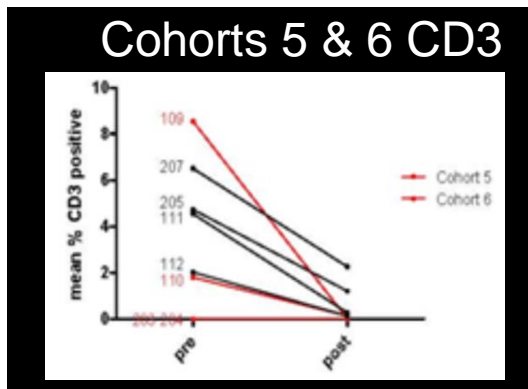
Eteplirsen: Substantial expression of novel dystrophin



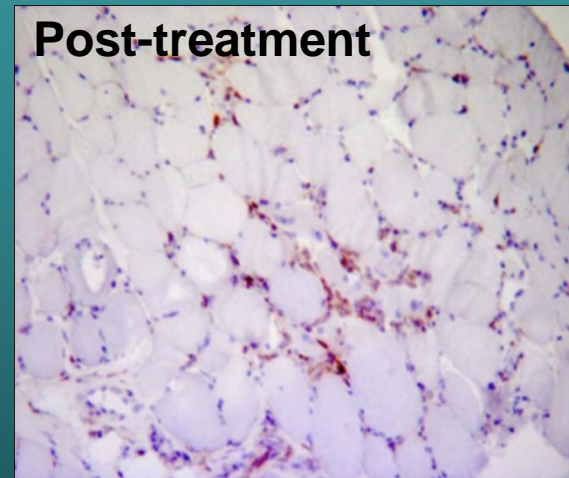
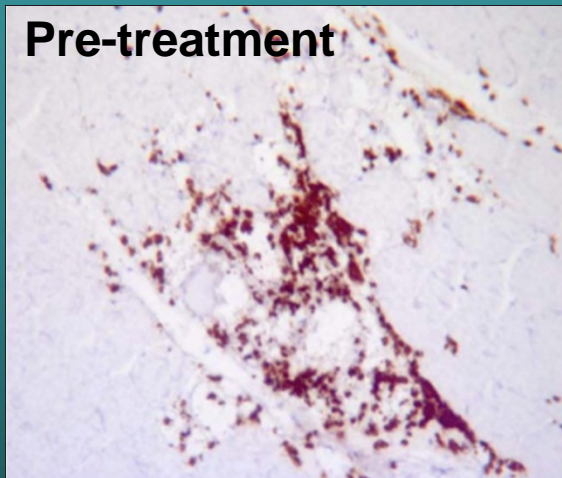
Sub-therapeutic cohorts (1-4) not shown.



Eteplirsen Dystrophin Restoration Associated with Reduction in Inflammatory Infiltrate



Inflammatory cells reduced post treatment



Antibody stained (brown) inflammatory cells



Eteplirsen: Demonstrated Proof of Concept

Key Phase 1b/2 Study 28 Conclusions

- **Well tolerated in all DMD patients**
 - Adverse events generally mild/moderate, transient and not drug related
- **Substantial and dose dependent novel dystrophin expression**
 - Up to 55% dystrophin positive fibers
 - Up to 27% dystrophin intensity per fiber
 - Up to 18% of normal protein expression on western blot
- **Reduction in inflammatory markers and no immune response**



Is it likely that dystrophin levels in biopsies can predict clinical outcome?

- Becker's Muscular Dystrophy is characterized by $> 5\%$ dystrophin levels. Even 10% dystrophin marks a milder form of BMD.
 - Mouse and dog models of DMD show a clear correlation of dystrophin levels with improved muscle performance, even at $20\text{-}30\%$ protein levels.
 - As in other diseases that lack an essential protein, recovery of even low levels of protein could result in significant clinical outcome.
 - Clinical studies are required to better understand the correlation of dystrophin levels with clinical outcome.
- AVI clinical study in progress: placebo controlled, will measure correlation of dystrophin levels with multiple clinical outcomes.