Scope of the meeting: Recent advances in drug development for Duchenne Muscular Dystrophy (DMD) present an opportunity for the development and validation of robust methods for the objective, reliable, and quantitative measurement of dystrophin and related muscle proteins. This workshop, cosponsored by the Food and Drug Administration and the National Institutes of Health was convened in order to discuss currently available methodologies and to identify scientific knowledge gaps and opportunities for improving dystrophin protein detection in the context of drug development.

Agenda

7:30 am  Registration

Opening Remarks

8:30 am  Christopher P. Austin, MD, Director, National Center for Advancing Translational Sciences, National Institutes of Health (NIH)

8:45 am  Richard A. Moscicki, MD, Deputy Center Director for Science Operations, Center for Drug Evaluation and Research (CDER), Food and Drug Administration (FDA)

8:55 am  Dystrophin: Convening the Conversation - Pat Furlong, President, Parent Project Muscular Dystrophy

9:10 am  Background and Meeting Objectives Related to Analytical Challenges and Opportunities with Dystrophin Methodologies - Ashutosh Rao, PhD, Division of Biotechnology Research and Review, Office of Biotechnology Products, CDER, FDA

Session I: The Dystrophin Gene and Protein – [Moderators Glen Nuckolls, PhD, Executive Secretary, Muscular Dystrophy Coordinating Committee, Program Officer, Neurogenetics Cluster, National Institute of Neurological Disorders and Stroke, NIH and Ashutosh Rao, PhD]

This session will provide a background on current understanding of the dystrophin gene and its various products and associated proteins as they relate to protein quantification methodologies and assays of dystrophin function in DMD and BMD.

9:30 am  Dystrophin Quantitation Using Full-Length Recombinant Protein as Standard - James M. Ervasti, PhD, Paul and Sheila Wellstone Muscular Dystrophy Center, University of Minnesota, Minneapolis, MN
Expression of Exogenous Dystrophin, Isoforms, Splice Products and Synthetic Constructs - Jeff Chamberlain, PhD, Departments of Neurology, Medicine and Biochemistry, University of Washington, Seattle, WA

10:00 am Break

Session II: Dystrophin Quantification Methodologies – [Moderators Glen Nuckolls, PhD, Ron Farkas, MD, PhD, Division of Neurology Products, Office of Drug Evaluation 1, CDER, FDA]

This session will focus on identifying key technologies, their limitations including detection and quantification sensitivities, linearity, reproducibility, and identified development and validation needs/hurdles for each method. Published and unpublished data using each method on human normal and disease samples will be discussed. Image and other data analysis, as well as statistical methods will also be discussed.

10:15 am Antibody Probes for Dystrophin Protein Detection - Glenn E. Morris, D.Phil., Wolfson Centre for Inherited Neuromuscular Disease, RJAH Orthopaedic Hospital and Keele University, Oswestry, UK

Immunohistochemistry/Fluorescence and Image Analysis

10:30 am Detailed Methodology of Dystrophin Quantification – Valentina Sardone, PhD, Institute of Child Health, University College, London

10:45 am Changes and Variability in Dystrophin Detection: Immunofluorescence, Antibody Staining and Imaging Methodologies - Chantal AC Beekman, Prosensa, Leiden, Netherlands

11:00 am Methodology for Quantification of Dystrophin Positive Muscle Fibers Using Widefield Fluorescence Microscope – Zarife Sahenk, MD, PhD, The Research Institute at Nationwide Children’s Hospital, Columbus, OH

11:10 am Assessment of Mean Fluorescence Signal Intensity of Muscle Fibers Expressing Dystrophin - Louise R. Rodino-Klapac, PhD, The Research Institute at Nationwide Children’s Hospital, Columbus, OH

11:20 am A Comprehensive Approach to Quantifiable and Meaningful Dystrophin Measurements in Clinical Trials – Peter Sazani, PhD, Executive Director, Medical Affairs, Sarepta Therapeutics

11:35 am Defining Standards for Dystrophin Quantification in DMD Muscle Biopsies - John Babiak, PhD, Senior Vice President, Drug Discovery Technologies, PTC Therapeutics, Inc
11:50 am Quantitative In-Situ Measurement of Biomolecules for Companion Diagnostics - David Rimm, MD, PhD, Yale University School of Medicine, New Haven, CT

12:05 pm Panel Discussion - Dystrophin Quantification by Histological Methods

12:45 pm Lunch

Session II continued – [Moderators Glen Nuckolls, PhD, Ashutosh Rao, PhD]

1:15 pm Dystrophin Quantification in Clinical Trials - Sebahattin Cirak, Dr. Med., Dipl Chem, Institut für Humangenetik, Klinik und Poliklinik für Kinder- und Jugendmedizin, Zentrum für Molekulare Medizin

1:30 pm Quantification by Western Blotting: Challenges and Correlations - Kevin Flanigan, MD, Center for Gene Therapy, The Research Institute at Nationwide Children’s Hospital, Columbus, OH, Professor of Pediatrics and Neurology, Ohio State University

1:45 pm Targeted Quantification of Dystrophin by Mass Spectrometry and Comparison to Antibody Based Assays - Yetrib Hathout, PhD, Center for Genetic Medicine, Children’s National Medical Center, Washington, DC

2:00 pm Variable Dystrophin Content within Myofibers, between Myofibers, and between Regions of Muscles: Determining the Level of Dystrophin that makes a Clinical Impact in BMD and Manifesting Carriers of DMD - Eric Hoffman, MD, Center for Genetic Medicine, Children’s National Medical Center, Washington, DC

2:15 pm Panel Discussion - Dystrophin Quantification from Tissue Extracts

Session III: Muscle Biopsies and Sample Handling [Moderators - Glen Nuckolls, PhD, Ron Farkas, MD]

This session will focus on muscle biopsy collection procedures (open and needle), anatomical site selection, guided versus non-guided biopsy, experiences with muscle biopsy handling and processing, and reference tissue banks.

2:45 pm Methods of Muscle Sampling - Rabi Tawil, MD, Professor of Neurology, University of Rochester Medical Center, Rochester, NY

3:00 pm Open Versus Needle Muscle Biopsy - Kathryn Wagner, MD, PhD, Center for Genetic Muscle Disorders, Kennedy Krieger Institute, Baltimore, MD

3:15 pm MRI/MRS Assessment of the Status of Skeletal Muscle in DMD Patients – H. Lee Sweeney, PhD, William Maul Measey Professor of Physiology, University of Pennsylvania School of Medicine, Philadelphia, PA

3:30 pm Break
Session IV  Facilitated Panel Discussion

3:45 pm  [Moderators - John Porter, PhD, Chief Executive Officer, Parent Project Muscular Dystrophy and Richard A. Moscicki, MD]

Panelists: Jeff Chamberlain, Kevin Flanigan, MD, Eric Hoffman, MD, Katherine Wagner, MD, H. Lee Sweeney, PhD

1. What is the current status of assay validation and methodology for quantification of dystrophin in the context of drug development? What are the key opportunities and knowledge gaps in the field?

2. What are the necessary next steps to advance the precision and reproducibility of technology to acquire, measure, and analyze the proteins that are associated with Duchenne muscular dystrophy?

3. What are the Action Items from this workshop that will continue to advance robust methods for the objective, reliable, and quantitative measurement of dystrophin and dystrophin-related muscle proteins?

5:15 pm  Closing Remarks - Ellis F. Unger, MD, Director, Office of Drug Evaluation I, Office of New Drugs, CDER, FDA

5:30 pm  Adjournment