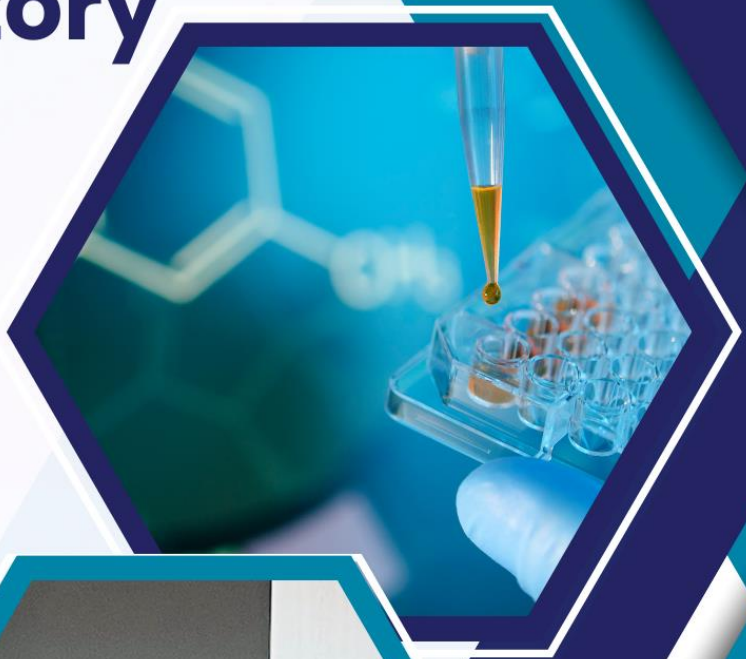
REAGAN-UDALL FOUNDATION FOR THE FDA

Qualifying Biomarkers To Support Rare Disease Regulatory Pathways:

Proceedings Summary



Denali Therapeutics, Orchard Therapeutics, REGENXBIO Inc., and Ultragenyx provided funding for this event

Qualifying Biomarkers to Support Rare Disease Regulatory Pathways

Case Example: Heparan sulfate in neuronopathic lysosomal storage diseases

This report serves as a high-level summary of the Reagan-Udall Foundation for the FDA's *Qualifying Biomarkers to Support Rare Disease Regulatory Pathways* public workshop held on February 21, 2024. This summary captures the essence of the workshop which offered valuable insights for scientific exploration. To view to view the full transcript, recording, and other meeting materials, visit ReaganUdall.org.

Biomarkers in Rare Genetic Diseases

Peter Marks, MD, PhD, Director, Center for Biologics Evaluation and Research, U.S. Food and Drug Administration

The use of biomarkers to develop treatments for rare genetic diseases is an essential topic that warrants serious consideration. Biomarkers are characteristics that are objectively measured as indicators of health, disease, or a response to an exposure or interventions, including therapeutic interventions. The U.S. Food and Drug Administration (FDA) has long utilized biomarkers in conditions like hypertension, diabetes, and cancer. Biomarkers play a crucial role in rare diseases where natural history data may be scant, providing reliable measures when clinical endpoints are challenging to establish due to disease diversity or slow progression. Biomarkers may offer insights into disease progression and treatment response, aiding in shorter clinical trial durations.

The use of biomarkers can be helpful in more traditional interventions and in gene therapies. More than 15 gene therapies have been approved for use in the US, and many therapies for rare diseases are in development. Leveraging biomarkers, where enzyme, protein, or metabolite levels can be measured, enables earlier prediction of clinical outcomes.

The FDA may also consider biomarkers in the accelerated approval pathway in which a drug is studied using a surrogate endpoint (which may be a biomarker) that is considered reasonably likely to predict a clinical benefit. Accelerated approval mechanisms hold promise in helping to address rare diseases, however, ensuring the accuracy and precision of biomarker measurements is paramount for successful application. Analytical method validation for biomarkers is a rigorous process involving assay design, qualification, and validation to ensure reliability and consistency over time and across different settings. Factors such as accuracy, precision, linearity, sensitivity, and stability of assays are meticulously evaluated. While biomarkers can be tailored to specific programs,

their broader qualification or validation can benefit the wider medical community. During the workshop, Dr. Peter Marks, Director of the Center for Biologics Evaluation and Research at the FDA stated, "If we don't lean into accelerated approval, we're going to leave a lot of patients behind, and we may even bring the field into a place where we have even more products dropping out of development."

To further explore the application of biomarkers for rare disease and accelerated approval, the workshop used Neuronopathic Mucopolysaccharidoses (MPS) as a case study.

Case Study: Understanding Neuronopathic Mucopolysaccharidoses (MPS)

Mark Dant, Founder and Volunteer Executive Director, Ryan Foundation
Joseph Muenzer, MD, PhD, Professor, Pediatric Genetics and Metabolism, University of North Carolina at Chapel Hill

To set the stage for the case study discussion, Dr. Joseph Muenzer (UNC Chapel Hill) and Mark Dant (Ryan Foundation) described the mucopolysaccharidoses (MPS) from the perspective of a health professional and an MPS parent. Dr. Muenzer described that MPS are a group of lysosomal storage disorders comprising 12 identified enzyme deficiencies across eight types. MPS are ultra-rare disorders. The prevalence of all MPS types in the US is estimated to be under 2,500 patients, with individual disorders likely affecting fewer than 500 individuals each. These disorders are clinically diverse and progressive, manifesting with both physical and central nervous system involvement predominantly marked by cognitive impairment and culminate in premature death among neuronopathic patients.

The diagnostic journey for MPS patients can be protracted, as symptoms may not manifest until early childhood, by which time a clinically diagnosed neuronopathic MPS individual usually already has irreversible brain damage. The biochemistry of MPS is well understood and cerebrospinal fluid (CSF) heparan sulfate (HS) is always elevated in individuals with neuronopathic MPS. CSF HS correlates with brain tissue HS in MPS animal models. FDA-approved treatment is available for somatic disease, but not the brain disease in neuropathic MPS. Reliance on clinical efficacy with placebo-controlled trials to demonstrate effectiveness results in irreversible brain damage in the control group.

Dr. Muenzer provided two examples of clinical trials that illustrate some of the challenges developing therapies for the MPS II brain disease. The first was a Takeda neuronopathic MPS II phase II/III trial studying monthly intrathecal administered of idursulfase-IT. The study did not meet its pre-specified endpoint. In a post-hoc analysis of patients less than 6 years of age, a significant p-value was observed on clinical endpoints, consistent with Dr. Muenzer's clinical perspective that younger patients appeared to have significant clinical benefit. CSF GAGs measured at that time using a nonspecific GAG assay (thrombin assay) demonstrated a significant reduction of CSF GAGs, but newer assays

demonstrate only ~30% reduction in CSF HS. The second example is the Denali Therapeutics ongoing phase I/II study with DNL310, a blood-brain barrier penetrating intravenous enzyme, which has demonstrates a normalization in CSF HS that is sustained over time, even in patients with high pre-existing antibodies. This study provides compelling evidence of biologic correction that is supported by the observation of a decrease in secondary cellular biomarkers like GM2 and GM3 and a profound reduction in NFL, a marker of neurodegeneration. Thus, with high sensitivity GAG assays and advanced science, therapeutics are in development that enable significant reduction/normalization of CSF HS and additional biomarker evidence that supports that CSF HS should be used for accelerated approval. Regulatory changes are needed based on scientific advancements using CSF HS as a biomarker in the accelerated approval pathway to bring treatments to individuals with neuronopathic MPS.

Ryan Dant, son of Jeanne and Mark Dant, was diagnosed with MPS I in 1991 at the age of 3. Ryan participated in a trial for enzyme-replacement therapy targeting MPS 1 in early 1998. The treatment aimed to replenish what Ryan's body couldn't produce, thereby clearing the accumulated stored substrate that was already wreaking havoc on his body. The drug was eventually approved, but not before the completion of two FDA trials and five full years passing. Since that groundbreaking trial for MPS I 21 years ago, MPS II, IV, VI, and VII all have an approved enzyme replacement therapy to treat somatic disease. All showed substrate reduction, yet all were forced to have a double-blind, placebo control arm in the trial. The challenges and frustrations for parents of children diagnosed with neuronopathic MPS disorders like MPS I, II, and III however, begin at diagnosis, with the realization that there are no approved drugs in the US or Europe which address their children's severe and progressive neurological disease. In these cases, the path towards approval has been clouded with uncertainty, thus contributing to limited drug development and unrealized treatments for this devastating group of diseases in children. Built upon the personal contributions of patients themselves who participate in research, scientific advances continue and are yielding greater clarity around the application of biomarkers in drug development. Our regulatory system must evolve with the science. The patient community feels the ultra-rare disease eco system is not aligned with the regulatory process and transformational science is being left behind.

Case Study:
Measuring Glycosaminoglycans (GAGs), including Heparan Sulfate

Maria Fuller, PhD, Professor, Genetics and Molecular Pathology, University of Adelaide

During this session, Dr. Maria Fuller (University of Adelaide) explained the measurement of glycosaminoglycans (GAGs), which are essentially sugar molecules, a type of carbohydrate, that are incompletely degraded in the MPS disorders and therefore accumulate. Their measurement in biological samples has long been a mainstay of laboratory diagnosis.

Traditional methods for measuring GAGs, such as urine dye-binding tests and electrophoresis, have limitations in terms of precision and specificity. However, the recent introduction of mass spectrometry-based platforms into the diagnostic laboratory offers improved sensitivity and specificity, allowing for quantification of specific GAG fragments. Consistent, high-quality testing of GAGs has enabled their use as biomarkers for diagnosis, prognosis, and treatment monitoring.

Case Study:
Animal Model Translation to Human Application

Nidal Boulos, PhD, CCRP, Director, Clinical Outcomes Research, REGENXBIO Inc.
Patricia Dickson, MD, Professor, Washington University School of Medicine, St. Louis
Matthew Ellinwood, DVM, PhD, Chief Scientific Officer, National MPS Society

Animal models can provide valuable insights into disease mechanisms and help researchers explore new therapeutic targets, particularly for rare diseases. Animal models used in MPS research don't just model MPS disease. They are true homologues of the human conditions, involving the same genes, the same enzymes, and the same substrates. Hence, these animal homologues closely mimic the human condition. They share similar genetic, physiological, and pathological profiles with the corresponding human syndromes. Studying MPS in animal models allows researchers to understand the disease's progression, to confirm basic assumptions regarding pathophysiology, to test potential therapies, and to evaluate safety and efficacy before moving to human trials. In this session, three speakers described findings relevant to potential interventions and biomarker readouts for MPS in animal models.

In her presentation, Dr. Patricia Dickson (Washington University School of Medicine, St. Louis) described her experiments to test whether CSF heparan sulfate levels might reflect brain heparan sulfate, or whether CSF heparan sulfate might instead be reflective of heparan sulfate in the serum. For these experiments she used a membrane-tethered alpha-N-acetylglucosaminidase (NAGLU) enzyme approach to restrict cross-correction, and administered this using adeno associated viral (AAV) vectors to MPS IIIB mice. She showed that when membrane-tethered NAGLU was delivered to MPS IIIB mice via intravenous AAV7 administration, which primarily targeted systemic organs but not the brain, there was no decrease in heparan sulfate in CSF or the brain. When membrane-tethered NAGLU was administered intraventricularly to MPS IIIB mice using AAV9 and a promotor that expressed the enzyme in brain neurons, heparan sulfate decreased to normal levels in the brain and CSF but the serum levels were unaffected. She concluded from these experiments that heparan sulfate levels in CSF reflect heparan sulfate in the brain and do not reflect heparan sulfate in the serum.

Dr. Matthew Ellinwood (National MPS Society) discussed a study that evaluated a compound in a prevention treatment model. Treatment with the compound yielded significant reductions in brain tissue GAGs and CSF heparan sulfate levels, particularly at a higher dose. Moreover, improvements

were observed in lysosomal volume, neuroinflammation, and pathological astrocytosis, along with prevention of cerebellar atrophy.

Dr. Nidal Boulos (REGENXBIO Inc.) introduced us to an investigational gene therapy (RGX-121) for the treatment of Mucopolysaccharidosis Type II (MPS II), also known as Hunter syndrome. RGX-121 is designed to address the underlying cause of MPS II by providing the missing enzyme needed to break down complex sugars (GAGs) in the body.

The development journey of RGX-121 for treating neuronopathic MPS II involved extensive preclinical and clinical investigations. During the preclinical investigation, treatment was administered into the CSF via intracerebroventricular injection of mice and investigators looked at neurobehavioral assessment in these mice using the Barnes maze tool. The Barnes maze tool is a measure of spatial learning and memory. Results showed that treated MPS II mice improved at escaping the platform while untreated MPS II mice did not. In addition, treated MPS II mice showed significant reductions in GAGs in CNS tissues, urine and peripheral organs indicating both a CNS and systemic benefit.

As part of RGX-121 clinical development program HS, the main GAG elevated in MPS II, was investigated as a key biomarker. Access to human CSF samples from neuronopathic and attenuated MPS II patients showed HS disaccharide D2S6 was significantly elevated in patients with neuronopathic MPS II. Furthermore, HS D2S6 concentrations in CSF distinguished neuronopathic from attenuated MPS II patients and was reflective of disease pathology.

RGX-121 is being investigated in a Phase 1/2/3 clinical study (CAMPSIITE™; NCT03566043) in neuronopathic MPS II patients to address the unmet need of CNS disease involvement while maintaining systemic benefit. The surrogate endpoint in this study is CSF levels of HS D2S6. Significant reductions in median CSF HS D2S6 levels of 86% were observed in CAMPSIITE pivotal phase as early as week 16 post treatment, with levels approaching normal in some participants.

Case Study:
Relationship Between Cerebrospinal HS Levels & Clinical Outcomes

Simon Jones, MBChB, Consultant, Paediatric Inherited Metabolic Diseases, Manchester NHS Hospitals and University
Heather Lau, MD, MS, Executive Director, Global Clinical Development, Ultragenyx
Eric Zanelli, PhD, Co-Founder, Allievex

Understanding the relationship between CSF heparan sulfate levels and clinical outcomes in MPS is essential for disease monitoring, treatment evaluation, and improving patient care and outcomes. During his presentation, Professor Simon Jones (Manchester NHS Hospitals and University) described a study on MPS IIIA Natural History. This study provided insights into the development of children with MPS IIIA, revealing significant challenges for clinical trial design and outcome

measurement. Participation required extensive assessments and lumbar punctures over a two-year period, and the study highlighted the heterogeneous nature of disease progression among affected children. While some participants followed a slowly progressing trajectory, others exhibited a more rapid decline in skills over time.

From a clinical trial perspective, the ideal trial population would be homogeneous, allowing for more accurate assessment of treatment efficacy. However, the small numbers and observed heterogeneity among MPS IIIA patients poses a significant challenge in selecting suitable candidates for trials. Additionally, determining the appropriate timing for intervention presents a dilemma. Treating patients early, while still within the normal developmental curve, may offer the best chance for preserving neurodevelopmental milestones. Conversely, treating patients at later stages, when symptoms are more pronounced, may lead to improvements, albeit with challenges as achieving a response once the brain is damaged is difficult.

According to Professor Jones, "the traditional approach of long-term follow-up with a placebo group is financially impossible and ethically inappropriate." Alternative trial designs and outcome measures are needed to advance therapeutic development for these devastating disorders.

Dr. Eric Zanelli's (Allievex) presentation delved into how the journey began with a 48-week natural history study, followed by an interventional study and its extension allowing to measure the clinical efficacy of an enzyme replacement therapy consisting of tralesinidase alfa, aka AX 250, over 240 weeks. Normalization of HS levels in both CSF and plasma within three to four weeks of treatment initiation demonstrated target engagement, with sustained normalization often observed for over five years. This normalization was attributed solely to the interventional treatment, as elevated HS levels were evidenced in both untreated subjects and subjects with treatment interruptions.

Investigation into cortical gray matter volumes by magnetic resonance imaging revealed an initial drop followed by stabilization, and often rebound, suggesting a protective effect on brain volume in all subjects consistently treated with AX 250. Notably, a correlation was observed between changes in cortical matter volume overtime and cognitive scores. Three out of four subjects who started treatment before 3 years of age had normalized CSF HS levels, brain volumes within normal range, and significant cognitive improvements after more than five years of treatment.

Dr. Heather Lau (Ultragenyx) presented the use of CSF HS as an early biomarker predictive of clinical outcomes based on a data analysis from August 2023 on Ultragenyx's UX111 program. UX111 is an intravenously administered AAV9 in vivo gene replacement therapy for treatment of pediatric patients with MPS IIIA. In an open-label dose-escalation phase 1/2/3 study, 17 enrolled patients were either 2 years old or younger, or greater than 2 years with a cognitive developmental quotient of at least 60 on the Bayley Scales of Infant and Toddler Development 3rd Edition (BSITD III) and received the highest dose of 3 x 1013 vg/kg. In this modified intention-to-treat (mITT) population, CSF HS exposure was calculated using time-normalized area under the curve (AUC) of percentage change in CSF HS substrate from baseline, which utilizes all available measurements for each patient

over the course of the study. There was a 63.3% sustained reduction in CSF HS exposure with a median follow up of 24 months. This reduction in CSF HS exposure was associated with reduction in toxic secondary storage markers (i.e., gangliosides GM2 and GM3), reflecting correction of lysosomal dysfunction. There was also stabilization of brain total cortical volumes, a late biomarker indicating preservation of neurons. Furthermore, there was a statistically significant correlation between reduction in CSF HS exposure and the estimated yearly change (EYC) in Bayley cognitive raw scores in the mITT population. For 15 of the 17 patients in this mITT population, there was both a reduction of CSF HS exposure of >50% and a positive rate of change in cognition based on the EYC in Bayley raw scores. Overall, these data support the use of CSF HS as a biomarker reasonably likely to predict clinical outcomes. Although these data focused on a younger population treated at the highest dose, all subjects in the study had a rapid reduction in CSF HS. Dr. Lau emphasized that cognition is only one measure of neurologic function and that they will evaluate the impact of UX111 on other developmental domains, including at the item level on these standardized assessments, to understand the potential benefit for all patients treated with UX111, especially the older patients. The benefit may be different but equally impactful for older patients with this fatal disease. At the end of her presentation, Dr. Lau concluded that the conference reflected a consensus among clinicians, scientists, patient advocates, and industry sponsors that the totality of preclinical and clinical evidence presented does support the use of CSF HS as a surrogate endpoint that is reasonably likely to predict clinical benefit in neuronopathic MPS.

Panel Discussion:
Challenges in Using Biomarkers to Support Rare Disease Approvals

John Crowley, JD, MBA, Executive Chairman, Amicus Therapeutics, Inc./ Incoming President & CEO, Biotechnology Innovation Organization

Cherie Fathy, MD, MPH, Medical Officer, Office of Therapeutic Products, Center for Biologics Evaluation and Research, FDA

Carole Ho, MD, Chief Medical Officer & Head of Development, Denali Therapeutics, Inc.

Gavin Imperato, MD, PhD, Chief of General Medicine Branch 4, Office of Therapeutic Products, Center for Biologics Evaluation and Research, FDA

Edward Neilan, MD, PhD, Chief Medical & Scientific Officer, National Organization of Rare Diseases

Cara O'Neill, MD, Chief Scientific Officer & Co-Founder, Cure Sanfilippo Foundation

James Wilson, MD, PhD, Rose H. Weiss Professor and Director, Orphan Disease Center, Professor of Medicine and Pediatrics, Director, Gene Therapy Program, Perelman School of Medicine, University of Pennsylvania

Moderator: ***Susan C. Winckler, RPh, Esq.,*** *CEO, Reagan-Udall Foundation for the FDA*

In the final session of the workshop, a broad array of experts discussed the potential—and need—for using biomarkers to support the approval of interventions for rare diseases and to leverage this experience for other diseases. The panel discussion focused on how converging data across basic biology, animal model generation and characterization, patient natural history data, and interventional studies in combination with collaboration across the drug development ecosystem and regulators can enable biomarkers to support rare disease approvals.

Discussion began with animal studies, which play a crucial role in advancing our understanding and treatment of rare diseases. These studies allow researchers to investigate the underlying mechanisms of these conditions, test potential therapies as well as identify potential new biomarkers, and assess their safety and efficacy before moving to human trials. Moreover, because rare diseases often lack sufficient human data, animal models provide valuable insights that can accelerate the development of treatments and improve outcomes for patients with these conditions. Dr. James Wilson (University of Pennsylvania) highlighted, "We used to think about animal studies as IND [Investigational New Drug]-enabling that allow the support of an IND. We now talk about them as BLA [Biologics Licensing Application]-enabling. So in other words, how can we structure those preclinical studies, which may mean just slight modifications of what you measure, so that when you complete those studies, you not only have safe to proceed to get into the clinic, but you position the program so that when you get to the point where, 'Wow, this is really looking well,' that you could leverage the animal studies to support that this biomarker would predict clinical benefit."

In addition, natural history studies play a crucial role in the development of effective treatments for rare genetic disorders. These longitudinal investigations provide valuable insights into the progression of diseases, elucidating the natural course of symptoms and variability of clinical manifestations among affected individuals. By systematically tracking the clinical, biochemical, and radiological characteristics of patients over time, natural history studies contribute to the identification of key disease milestones, the assessment of disease burden, the identification of potential endpoints in clinical trials, and the prediction of clinical outcomes. Moreover, they facilitate the stratification of patients based on disease severity and genotype-phenotype correlations, which are essential for optimizing patient management and designing targeted interventions. As panelist John Crowley (Amicus Therapeutics) observed, "If we're going to be doing these studies where placebo controls are impractical or oftentimes largely unethical, we're going to need to have robust natural history studies. When parents and families call me and they ask, 'What can we do?' particularly in these diseases where there is very little research in an advanced stage going on, I always tell them two things. One, educate the community, find more people like you… And then the second thing is, work with your…whole ecosystem to build those natural history studies because that's what ultimately I think is going to be an incredibly powerful tool for us doing these studies."

From a regulatory perspective, using biomarkers for rare genetic diseases presents a myriad of challenges due to the unique nature of these conditions and the limited understanding of their underlying mechanisms. The rarity of these diseases equates with small patient populations, making it challenging to collect sufficient data to establish the reliability, validity, and clinical relevance of potential biomarkers. Panelist Dr. Cherie Fathy (FDA CBER) observed that evaluating a biomarker requires collating multiples sources of evidence and opportunities for scientific exchange that allow for discussion and exploration of those various sources of evidence. Furthermore, the heterogeneity

observed within these patient populations, including variations in disease presentation, progression, and response to treatment, complicates the identification of biomarkers that accurately reflect disease status and treatment outcomes across diverse subgroups.

Panelist Dr. Gavin Imperato (FDA CBER) explained three categories of challenges for regulators: evolution, collaboration, and communication.

Regarding evolution, there's been an explosion in basic science that has been rapidly translated to the clinic in the setting of many diseases. That evolution presents a challenge from the regulatory standpoint because that advance has happened so quickly. "How do regulators deal with the unique regulatory challenges that are presented by advances that are wonderful, products that clearly have potential by virtue of available data, but don't fit squarely into a known regulatory paradigm?"

The second category is collaboration. Engaging all the stakeholders in the drug development ecosystem is critical. Regulators gain significantly by interacting with patients and listening to patients and caregivers. "It was really helpful to hear some of these patient stories earlier in the day because that comes to tremendous benefit." Because the evidentiary framework for biomarkers and accelerated approval is holistic, interactions among different disciplines is essential; it's essential for clinical reviewers to understand and engage with pharmacology and toxicology colleagues who are evaluating data from disease-relevant animal models.

Finally, open communication is important—within a regulatory agency and with industry sponsors.

MPS represents a poignant example of an unmet medical need in the realm of rare genetic disorders. Challenges include limited efficacy in addressing neurological manifestations, variable responses among patients, and the high cost and complexity of current treatments. Dr. Edward Neilan (NORD) noted, "For rare genetic metabolic disorders [such as those discussed in the case study], where we know the next few steps towards the pathogenesis, this really seems like a prime area to use biomarkers and accelerated approval and get it right many more times than you get it wrong."

Addressing this unmet need requires collaborative efforts among researchers, healthcare providers, regulatory agencies, and pharmaceutical companies to accelerate the development and approval of novel treatments that offer improved outcomes for individuals living with MPS and their families. These same needs apply in other rare genetic diseases. Dr. Cara O'Neill (Cure Sanfilippo Foundation) poignantly expressed:

> The accelerated approval path is here for us, and I think this has allowed us a chance to really talk about how we can move forward in that. In accelerated approval, naturally uncertainty is going to be a component of that, but I think we take a step back and understand what we do know for certain because there's a lot more that we do know than we don't know. And what we do know is that this disease causes unrelenting losses of every skill. And you saw that in the videos this morning. Our kids go from singing their ABCs to utterances, stuttering, and then silence. From enjoying their birthday cake to being fed through a gastrostomy tube

in their stomach. Running wildly on the beach through the streets where you can barely keep a hold of them to being unable to move and even roll over in bed. They lose the ability to engage with us and the people that love them most. Our children become locked in and lost to us even though they are right there in front of us.

The massive harms of living with diseases like this must be weighed heavily in regulatory calculations of risk/benefit. Dr. Carole Ho (Denali Therapeutics, Inc.) echoed this sentiment, while also highlighting the evolution of the science in delivering highly sensitive and specific assays, greater understanding of the biology of disease, and increasing experience with clinical trial design in these rare diseases stating, "I think as we look at the science and we look at where we are today, we are ready to use this pathway for accelerated approval, and we need to apply this and move right away."

To view the recording, full transcript, and other meeting materials, visit ReaganUdall.org.