Qualifying Biomarkers to Support Rare Disease Regulatory Pathways
Case example: Heparan sulfate in neuronopathic lysosomal storage diseases
Hybrid Public Workshop
February 21, 2024 | 10am-4pm *(eastern)*

Morning Transcript

Welcome & Opening Remarks
Susan C. Winckler, RPh, Esq., CEO, Reagan-Udall Foundation for the FDA

Susan Winckler: It's 10:00 AM Eastern time and so it's time for us to go ahead and get started. So I want to welcome everyone who is in the room and those of us who are joining virtually. For those of you who are in the room, we are a little cozy, but we'll have an intense and hopefully a productive discussion for the rest of today. For those of you who I have not yet met, I am Susan Winckler and I serve as Chief Executive Officer at the Reagan Udall Foundation for the FDA and we are so pleased to be hosting this important workshop today. We are going to be digging into the topic of qualifying biomarkers to support rare disease regulatory pathways. Before we begin, I have to do the obligatory housekeeping issues. So many of our speakers are gathered here in Washington DC as well as I noted a few in-person participants, we have a significant number of virtual participants.

So if you see the speakers in me turning to the camera, it is just to make sure that those who are outside the room feel as welcome as those who are inside the room. Because of the size of the meeting, the virtual attendee cameras and microphones remain off throughout the event. And our in-person engagement will be primarily with those who are on the dais. We do have one planned virtual speaker, which is Dr. Dixon in the afternoon session, and then Dr. Imperato from FDA has fallen ill, but he will join virtually, so we'll still have his participation just not in person. We do want to hear from each of you and particularly through the Q&A session. So if you're online, use the Q&A function and I'll be able to see those questions. If you're here in the room, you should have received index cards with your name badge.

Go ahead and fill those out and hand them to a foundation staff member and I will work those into our dedicated question and answer sessions. Note that we are recording the meeting and as is a practice with all foundation public events, we will be posting the recording a compiled slide deck and the transcripts on the foundation website, which is reaganudall.org, in the next week or so. One note

for those of you who are in the room, if you all jump onto the Zoom, you will crush the wifi and then we won't have virtual attendees, so let's not do that. So just an acknowledgement that we have to recognize that. I also want to thank our sponsors. We would not be here today if we did not have support from Denali Therapeutics, Orchard Therapeutics, REGENXBIO and Ultragenyx, and we greatly appreciate your support and insight.

So let's think about our time together for the agenda. Think about this meeting in two components. We have an opening and a closing session that speak to the broader topic of qualifying biomarkers for rare disease regulatory pathways, and then in the middle to help us think through that higher level conceptual topic, we're going to focus with that case study in thinking about the application of a specific potential biomarker in rare disease, specifically heparin sulfate in neuronopathic mucopolysaccharidosis. Now in just a minute, I will turn the podium over to Dr. Peter Marks of FDA's Center for Biologics Evaluation and Research who will open us with the regulatory perspective on the criteria for qualifying a reliable biomarker in rare diseases. Then we'll move through the case study in a different session, three different discussions there, and then we'll close with a panel to bring us back out to that broader perspective.

So we're about to begin our meeting of scientific inquiry and exploration. This is not an advocacy event nor a decision-making event, but rather one where we can explore and better understand various topics. We have a packed day with an efficient 30 minutes for lunch and a break and one short break in the afternoon. We have the full agenda on the website and in the handouts. And with that, it is time for me to step out of the way and I'll ask Dr. Marks to come to the stage. Many of you are familiar with Dr. Marks, if not in person in many other experiences. He is the director of the Center for Biologics Evaluation and Research at FDA, a position he's held since 2016. Dr. Marks, you may take the podium, you may stand wherever you like.

Dr. Peter Marks: That sounds great.

Susan Winckler: We are excited to hear your presentation.

Biomarkers in Rare Genetic Diseases

Peter Marks, MD, PhD, Director, Center for Biologics Evaluation and Research, FDA

Dr. Peter Marks: So let me thank you first, I just want to say some thanks first. First of all, thanks so much to Reagan Udall for hosting this. Susan, thank you and your staff for setting this up, which really helps us tremendously because we don't always have the bandwidth to put these kinds of things together, and I think this was really important to happen timely and so they made this possible. I also want to thank our Office of Tissue, sorry, nearly saying the old name, the Office of Therapeutic Products at FDA. The Office of Therapeutic Products because their work went into getting this set up as well. I want to thank all of you here in the room and online for taking the time because I think this really is an important

discussion about thinking about biomarkers in the larger sense as well as having a discussion about one particular example.

So I think we'll talk about biomarkers in rare genetic diseases. I think it becomes quite important for reasons that I'll talk about that we really give this some serious consideration. Obviously, we've had the ability at FDA to use biomarkers for a long time. We use validated biomarkers constantly in hypertension and blood pressure. But oftentimes this has become more controversial in practice as we talk about specific applications. So let's kind of dive in here.

Why we care about this so much at FDA in the Center for Biologics is because it's interesting, people talk about needing a rare disease center at FDA. If you look and you look across our products at the center, about 60 to 70% of the products we regulate are for rare diseases. So rare disease products are important, and if you think about rare diseases in aggregate, the NIH, every time I go to an NIH meeting, the number of rare diseases has increased over the past few years.

Now are up to over 10,000 rare disorders affecting millions of individuals in the United States. So in aggregate, these are really important and I think that as I think about gene therapy moving forward, if we can't get it right in the rare disease space, we're never going to be able to get it right in larger populations, so important group.

I put this up here to say that we now have 17 approved gene therapies in the United States. You could make the case that there's maybe 18 because the exagamglogene got two indications both for sickle cell and for beta thalassemia. This is to shake you up a little bit because you probably don't recognize any of these products because it's all of their INN names, their generic names, which we don't usually use. But I was recently at a conference where, because of CME, we had to use these. So these are the names that are too long actually to put on one slide, but it is impressive that we've moved along here. But if we want to really for whatever's on the slide from my perspective, the reason why we're here today is in various stages of development across the United States and across the globe there are a lot of gene therapies for rare diseases that are almost there, and it's a question of how do we get them across the finish line? And I think some of the way we're going to get there is through use of biomarkers and our accelerator approval provisions.

There are a lot of things we could talk about today in terms of challenges to gene therapy, why gene therapies are falling out of development left and right in the United States. I'm just going to focus today on one which is clinical development timelines because that's what this lever of thinking about use of biomarkers and accelerated approval helps address.

Biomarkers help address some of our challenges in rare diseases, which if you don't have a lot of natural history data on a rare disease, it can be challenging to find a clinical endpoint that will be useful and biomarkers can help stand in there. Sometimes we have so much diversity in disease manifestation in what

we see in the clinic that biomarkers might potentially be more reliable in terms of measurement. In some cases, the time course of illness in terms of finding clinical effect is so long that you might see something first with something that you can measure. And in some cases it's really hard to see things in short periods of time, the time we usually do clinical studies over the course of six months or a year because you're looking at diseases where the manifestations are not reversible and so you're trying to detect lack of further decline. There again, biomarkers change may be something that can help us there, and I'm sure we could add to this list if we had time, but this is just the idea here that biomarkers are important to us.

They're very important if we're going to try to leverage accelerated approval. Biomarkers and intermediate endpoints, intermediate clinical endpoints allow us to leverage our accelerated approval provisions and particularly for gene therapies where we can measure enzyme activity levels, structural protein levels or metabolite levels. Think of what we'll be talking about today. It allows us to potentially have a handle on something that is reasonably likely to predict clinical outcome. Now we have to make sure that we make that connection and that's what I think some of the discussion we'll be having today is about. But if we can make that connection either in an animal model or in a human condition, we can use this provision and hopefully speed up getting products to patients with the knowledge that we do eventually have to get clinical evidence that these products have effect, but potentially with the ability to get products to patients much, much sooner.

And this is just to say that there are probably, because of time I don't have the whole presentation of the different scenarios ranging from the easiest low hanging fruit with biomarkers to the most difficult. But in a simple situation, if you have a gene therapy product which you can measure and measurement of that correlates with alleviation of the disease either in an animal model or you can show that levels at a certain value in humans are not associated with pathology, you have a way of having something that's reasonably likely to predict. It gets a little more complicated when we're dealing with metabolites and it gets even more complicated when we don't have metabolites. So there are various levels of complexity here and we'll have to deal with them as we move across all of the spectrum of rare diseases. But this is a handle on being able to move rare disease therapy development forward without some of the issues of waiting for clinical endpoints.

And we're very committed to trying to move in this direction. This means though that we end up having to put a lot of weight on these biomarkers and they really are critical in this accelerated approval pathway, whether or not they're directly connected or indirectly connected, and ultimately the accuracy and precision of measurement become really crucial things for us to think about. And over the next 10 minutes, I'm trying to use less of my time, I'm trying to move ahead, I've always been told stay ahead of schedule if you can. I'm going to try not to put people to sleep by talking a bit about how we go ahead and look at and think about biomarkers in terms of making sure they meet what

we would consider our standards for trying to use them for an accelerated approval. And I have to thank our office of regulatory operations that concentrates on this for providing me with some of their thoughts and slides.

So there are various stages of analytical method validation because when we're thinking about biomarkers, we're thinking about measuring something. When you measure something, as we'll come to there's how close to reality your measurement is and also then how well you can measure it from time to time. And those things hopefully are related, but they're not always related. So we have to think about designing an assay that can measure something successfully, showing that that assay actually does what it's intended to do, and then showing that it can do that over the course of time in a variety of settings. And so it's assay design, assay qualification and assay validation.

Assay qualification is that the assay is suitable for its intended purpose. Assay validation, it's suitable for its intended purpose on a routine basis. And really it's a matter of showing that the data that comes out of the assay using the specific assay that you have in place is relevant, it works correctly and gives you expected results, and it meets a certain performance criteria. And ultimately you want something that's validated so that you can get consistent results with that assay.

Validation is a matter of having analytic validation and clinical validation. Ultimately, for many of us, it's clinical validation which matters, right? We want to make sure that the biomarker links to the process that we're measuring and that it's relevant to the condition that we're considering. But we also have to start with analytic validation, which is that there is accuracy, precision, and reproducibility of the test so that we know that it's really measuring what it claims to measure. And having spent some time in the lab, you would think that these are easy things to make happen, but it can be more difficult than it seems at first glance. Ultimately, something that's on the slide that really has to be thought about is we need to also think about as we develop these, what are the implications? And as you see, our risk-based approach towards these biomarkers, it's what happens to something you get a wrong result, what will the implications be for the patient? And that's part of the analytical validation process.

So analytical assay validation explores a lot of different things, sensitivity, accuracy, precision, reproducibility. Is the assay linear? Can you dilute analytes to get to linearity? The range, the sensitivity and stability, how robust is it to various perturbations? Sometimes today we are having to validate software that goes along with these assays and sometimes we have to have cross validation of these assays. So a lot goes into this. I'm not going to bore everyone by going through each of these on a separate slide, but we have to think about this because it really is what goes into this. And as we come to my last slide, it's why a lot of planning to go into development programs.

Just for some pretty standard terms though, and I'm sorry to bore those who are very familiar with these. Accuracy, when we talk about the accuracy of something, does this really reflect reality, the closeness of a measure value to a standard or known value? And that could be a reference standard, reference controls, clinical truth, ground truth or reference labs measures. This basically is something that can be really challenging. I know from early days I kind of cut my teeth in industry in the days when we first had BCR able inhibitors, and it turned out that if you had the same sample and sent it around to different laboratories, you could get values that were several orders of magnitude difference. That's a problem because it was a reference standard, and so we actually knew what ground truth was. So accuracy is important.

Precision is basically within a laboratory how often do you get the same result? I can tell you from having run a diagnostic lab that it was very frustrating sometimes when you'd have a wild variation in a result, that's another issue. So it's really, precision can read reproducibility or repeatability. That's also important because even if you have something that, remember the precision, they're not absolutely linked, you can have a very precise measurement that's inaccurate and vice versa. This is something that we have to think about.

Linearity is an issue for us in some of these observations because for some analytes, you might not have a linear relationship in terms of the results you get across the entire analytic range. I'm not going to belabor this, but it is something we have to think about.

And then there's this issue of there are a lot of interfering substances that go around and you need to be able to actually detect what you need to when there are other potential interfering substances or other analytes in the sample matrix.

And then there are some other things that we talk about in terms of assay sensitivity, in terms of what you have in the background, the limits of detection and the limit of quantification.

And finally there's an issue that actually as a hematologist, this turns out to be one of the ones that's always been, I've watched this be a nightmare for us, especially early on, is the stability of your analytes, because in some cases, some of the things we measure are not necessarily stable and the samples may have to be processed in order to be stable, and the reagents themselves may or may not be stable or the controls. So we have to also think about stability.

Ultimately, I'm talking through all of this because as we think about this, I've talked about all of this because we have to think about this from really early on as we are thinking about developing an assay for use, and this is whether or not you're going to develop an assay for use that will be qualified, an assay that will ultimately be validated even for use in a single drug development program one needs to be starting early on with thinking about how you're going to get the necessary information to be able to have an assay that can be applied to be able

to look at the biomarker over time. And one of the things that sometimes comes up is this is what one needs to do for any given biomarker and biomarkers can be company specific, it can be program specific.

So a biomarker that you're developing does not have to be broadly applicable outside of your own development program. If you'd like that to be that's what's why we have a biomarker qualification program, and I'd encourage people, the Center for Drugs and the Center for Biologics have a biomarker qualification program. There's even a grant program for essentially drug development tools where you can develop a biomarker across programs. So that is potentially making something that could be useful for the broader community. And then there's validated biomarkers, validated biomarkers, take it yet another step further because those are a case where potentially using those, we can give something a traditional approval rather than a full approval. So there are a variety of terms that get thrown around here.

Biomarkers in general though can be when we don't say they're qualified or validated, they can be essentially unique to a given clinical development program, in which case they have to, all of these things when it can get confusing because we'll say you have to validate your biomarker. We're not saying you have to have a validated biomarker in the larger sense, what we're saying is you have to do the correct testing to show that you have the right analytical accuracy and precision and other things.

So the reason why this is also important is because from our center's perspective, we are very, very much focused, and you'll see you may meet a number of people from our center here, our office director of the Office of Therapeutic Products, which is now a super office, Nicole Verdun. Everyone's very committed. They're very aware of the challenges in rare disease gene therapy development, in fact, rare disease therapy development in general, but particularly in gene therapy development and the importance of biomarkers here. And I think we all see the need to be able to lean into the use of biomarkers to help us lean into accelerated approval to help move things forward.

I personally, just not speaking for the office, but I find that 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, which could potentially benefit patients. So we're looking to really lean in here to encourage people to engage with us around biomarker development, use of accelerated approval. I do not view, I said this across the circle last week, so I'll say it on this side of the circle this week. Biomarkers are really important to us. Accelerated approval is very important to us, and it's not really something that I would ever want to consider a dirty word. Accelerated approval is going to be the norm, I think for a lot of our approvals, at least our initial approvals of gene therapy.

So with that, I think I may have gotten done a little early and I'll turn it back to you, Susan, or we can take some questions, whatever you'd like.

Susan Winckler: Let's do some questions because I've been watching some come in on the chat and moving through. And let me, first, we know that you speak from the CBER, Center for Biologics perspective, but I would imagine, as I heard you speaking about linearity and specificity and sensitivity and those things, those aren't questions that are unique to CBER'S work?

Dr. Peter Marks: So I should have said this upfront, right? So hopefully my colleagues from CBER and my colleagues even from CDRH, well, especially since CDRH provided some of the background for those slides. Hopefully we'll all agree that those are things that we need in general for our assays.

Susan Winckler: To that point, I know a bit from my time at the agency that sometimes there's more coordination between centers and programs and sometimes less than the public would think about. What do you think about, tell us a little bit about do the relevant review teams at CBER and CDRH collaborate on rare disease topics?

Dr. Peter Marks: So there has been collaboration on rare diseases at FDA over the years. I think what we're seeing now is now that rare diseases are coming even into greater focus, you will see further collaboration between our centers. I think, is it perfect yet? I'm not going to be the one to say it's perfect yet, but I do think we're increasingly having conversations across centers for the first time. Over the past months, we've been having a regular meetings across CBER and CDRH to try to make sure that we're as well aligned as we can be. There will sometimes be differences between the centers because development of a small molecule and development of a gene therapy may have different considerations. That said, we're going to do our best to make sure that there are minimal differences. And when there are, we'll try to make sure we can explain why they exist.

Susan Winckler: And there are slightly different regulatory structures. Is that fair?

Dr. Peter Marks: Well, there's a difference between the structure of biologics is slightly different than for drugs, but I think the issue is that it's almost less about that and more about the fact that when you think about benefit risk considerations, if you have a small molecule that has reversible side effects that you can decide to give for a period of time, and if it doesn't work, okay, you move on to the next drug. If you have one AAV gene therapy in development for a rare disease and there's nothing else in development for it, you decide to give a child that AAV gene therapy, they're not going to be able to get some other, something else comes along for a little while that's an AAV gene therapy, that's not going to be available. And so it's even more of an issue if you have multiple AAV gene therapies for a given condition.

So you have to think about the fact that for some of the therapies that we're administering at CBER, it's once and then people make antibodies. And although people are trying to develop ways of getting around antibodies to AAV, at least at this point in time, it's not really a simple thing. So I think that's part of what goes into this. Also, the other issue, just another one is that small molecules, reversible effects, something that integrates into the wrong place into your genome, that's a lifetime event. So there are different considerations as people think about this, and I'd encourage people as we come to the panels later, they may be able to talk more about those.

Susan Winckler: Yeah, yeah, really helpful. Thank you. And you mentioned this, so I want to ask. There was a specific question about formally qualifying a biomarker, and so I think there's the capital Q, qualifying a biomarker, and then there's maybe small u, using.

Dr. Peter Marks: So we have a biomarker qualification process where you can propose a biomarker to be qualified. The agency will work with you to go through what needs to be done to get that qualification done. And once it's qualified, it can be used across different programs rather than for a single drug development program. So it actually, it's great because it helps, it floats all boats, but it's not the path that everyone takes, and it does take some time and effort both from the sponsor who's developing it as well as from the agency.

Susan Winckler: Absolutely. Are there examples of biomarkers being used for accelerated approval that did not go through that capital Q, qualifying?

Dr. Peter Marks: Yeah, so there are plenty of biomarkers that have been used over the years, in fact so many that I can't even, if you think about even in the influenza vaccine world, assays for immunogenicity that were developed at a company, they're unique to a company, they're not used cross companies so they're all over the place, that basically it's okay, right? As long as you can show that you have something that's a biomarker that can be measured accurately reproducibly, we don't necessarily always need all of the additional work that goes with showing, that gets to qualification.

The issue is if you're measuring something in a small group of labs or in a single lab, it's very different than if you're trying to measure something in many different labs. As I noted, I wish I had, I should have shown you, it is a great slide. It shows you about 20 or 25 labs that had samples, the same sample sent to them from CML patients, and we knew what the number of BCR able copies were, and they're all over the place. That's a problem if you're going to have a validated assay. Now today, here we are 15 years later from when that was made and now you'd see that pretty much every... Within very small fractions, 10%, because of the new genetics, people get the same number and there's not that kind of variation. That's how you get towards qualification. So you don't have to be qualified, but it's nice to be qualified.

Susan Winckler: And regardless, there are all of the elements I started to count and just the things that you consider that.

Dr. Peter Marks: But you still have to consider all those things.

Susan Winckler: Right.

Dr. Peter Marks: It's just there are some things that if you are in a single program, you don't have to worry about in the same way if you don't have to have multiple different laboratories. Reproducibility among different laboratories is not the same issue if you have a single laboratory that's doing it for a given program.

Susan Winckler: Yeah. Yeah. Great. Well, I would say, I'm going to have you underscore it again because it was probably in 80% of the questions that I received about just the... Are there differences between CBER and CDER and you answered this, that there are differences in the product. So anything else you want to say about that? As I think about it. Yeah.

Dr. Peter Marks: I think people like to accentuate differences and in any organization, we're going to have some differences because we value and we encourage the scientific independence of our reviewers and hopefully as it bubbles up to management, that helps get to some level where we have better harmonization between the different centers. I think increasingly, you'll see us working kind of hand in hand. I think, again, it may not be perfect yet, but I think the goal is to have us working as closely as we can so that when you get an answer from CDER or CBER, unless there's some good reason why we should have something that's different in how we do something, the answer should probably be the same. And will we get there tomorrow. No, but it shouldn't take five years to get there. My guess is this is something that over the next year or two or three, you'll see us gradually meld into something that's better harmonized.

Susan Winckler: Yeah. With the recognition that you are also dealing with different products.

Dr. Peter Marks: That's what I'm saying.

Susan Winckler: You're going to have different questions. Right.

Dr. Peter Marks: You're going to have differences, but we hope to be able to explain when there are differences.

Susan Winckler: Yeah. That's why there are two different centers.

Dr. Peter Marks: That's correct.

Susan Winckler: Because you have different questions to review.

Dr. Peter Marks: That's right. That's correct. Yeah.

Susan Winckler: All right. Dr. Marks, I will say in a brilliant regulatory fashion, you've put us five minutes ahead, which our next set of speakers are going to really appreciate. Let's thank Dr. Marks.

Case Study: Understanding Neuronopathic Mucopolysaccharidoses (MPS)

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

Susan Winckler: So we'll step back from that broader presentation and start our discussion into the case study. So our first, we want to set the stage here and talk about neuronopathic mucopolysaccharidosis, and so we have two speakers who are going to help us do that.

We will kick off with the distinguished gentleman right behind me. So Dr. Muenzer, thank you for joining us from your role as professor in the Department of Pediatrics and the Department of Genetics at the University of North Carolina in Chapel Hill and as director of the recently created Joseph Muenzer MPS Research and Treatment Center. We'll turn to you to give us this baseline presentation. I will give you the warning that when you see me come back here, you have 60 seconds left. I don't have playoff music, but it's pretty close.

Dr. Joseph Muenzer: I'm going to take five minutes of Peter's talk.

Susan Winckler: Maybe. I get to decide that, but yes.

Dr. Joseph Muenzer: I think the message I have is important. So one, I want to thank the Reagan-Udall Foundation for inviting me to speak really on behalf of the academic community and the patients who I have represented for the last 40 years. I'd like to briefly tell you who I am. I'm a physician scientist trained as a pediatric biochemical geneticist. I have greater than 40 years of experience in the diagnosis, management, and treatment of patients with mucopolysaccharidoses that were going to refer to as MPS.

Years ago, I created a mouse model for MPS II to help develop the treatment for Hunter syndrome or MPS II. I have greater than 50 MPS-specific publications on MPS. I've been involved in a PI for greater than 20 clinical trials for MPS over the last 20 years. I've seen over 500 neuronopathic MPS patients in my career, which is probably the most except for a few other centers in the world, as you'll hear from Simon Jones later today. I know this disorder. I've seen too many deaths in this ultra-rare group that are now preventable based on the recent scientific advances that continue to suffer and die prematurely because of the lack of regulatory flexibility. My disclosures are shown here.

My presentation will highlight that biochemistry and the clinical features of neuronopathic MPS, the treatment challenges for the brain disease in these

disorders, and the need to use CSF heparan sulfate as a biomarker for accelerated pathway to prevent another generation of MPS patients suffering irreversible brain damage and premature death.

So the MPSs are a group of lysosomal storage disorders. There's 12 known deficiencies, eight types. The hallmark of these disorders are really the increased urinary excretion of the partially degraded glycosaminoglycan fragments due to the primary event of the inter-lysosomal GAG accumulation. MPSs are not common. They are ultra-rare disorders. I estimate the U.S prevalence of all the MPSs is less than 2,500 patients, and each individual disorder is probably less than 500. These are heterogeneous clinically progressive disorders and clinically characterized by both physical or somatic involvement and/or central nervous system primarily with cognitive impairment with premature death in almost all of these neuronopathic patients. We'll talk about more in a little bit.

This just shows you the major clinical manifestations, which I won't go into. I could spend the next three hours talking about this one side, but I won't because Susan won't let me. In general, MPS patients appear normal at birth and subsequently developed somatic and cognitive impairment, but it's hard to recognize these and they go a long time before they clinically get diagnosed.

Nomenclature has always been challenging. Historically, Hurler syndrome equaled MPS. Then there was a Hunter form of Hurler syndrome and then in the 1960s, [inaudible 00:39:39] came along and came up with a much better classification as shown here on this slide. At that time when you classify, there wasn't hyaluronidase MPS IX or X or arylsulfatase K, but those are now part of the disorders. The nomenclature has always been challenging for the clinical and has evolved. Initially, MPS patients were described as having either severe or mild disease. About 20 years ago, I first heard Ed Wraith, Simon Jones, distinguished colleague and mentor, who used the term severe and attenuated to better describe the clinical spectrum in MPS in 2015.

I actually used the first time the term neuronopathic to describe the individuals with progressive cognitive impairment and non-neuronopathic to sort out because all these patients with MPS II have clinical somatic disease, but some have brain disease and some don't depending on... And in contrast you'll hear Sanfilippo patients only have the primary brain disease and very little physical disease.

The biochemistry is well understood in these disorders. These enzymes are involved in reducing a particular component of the non-reducing end. You'll hear more about this in terms of what that means from Dr. Fuller, but it's well understood for all these enzymes what happens. Everyone is a hydrolase except for Sanfilippo C, which is an acetyltransferase.

What are the lysosomes? The major function is to break down and recycle molecules and organelles into basic precursors. The body does a great job of

preserving crucial substrates. A defect in activity of lysosome enzyme results in either non-degraded or partially degraded substrate, and typically expansion of size of a number of lysosomes, which gives you lysosomal dysfunction. In the MPS disorders, resulting inter-lysosomal accumulation of GAG storage results in cell tissue and organ damage and clearly causing death of cells.

Here's an example of a white cell showing the increased lysosomal accumulation. That cell can't function as it should with this distended. Virtually the whole cell is filled with lysosomes that are distended. They look clear here because the substrate washes out during the fixation process.

What's the pathophysiology? The amount of residual enzyme activity is a primary driver of clinical severity. It's important to note that major classes of glycosaminoglycans are not evenly distributed throughout the body. Heparan sulfate is primarily in the brain. Keratan sulfate, somatic or physical. Dermatan sulfate there and keratan sulfate is primary a bone GAG.

The urinary GAG excretion just demonstrates that MPS I, II, and VI all accumulate dermatan and heparan sulfate. MPS III or Sanfilippo will only accumulate heparan sulfate in the urine where in contrast, VI accumulates dermatan sulfate and IV accumulate keratan sulfate. All the MPS orders that have progressive cognitive impairment, neuronopathic have elevated urine and CSF GAGs. No exceptions.

The unique glycosaminoglycan storage for each disorder has a wide range of clinical severity resulting from a variety of secondary events result in a complex cascade of cellular pathway. We don't need to know all the reasons. There's lots of reasons why GAGs cause clinical disease. We know that the defect in a gene results in a deficient enzyme. That deficient enzyme results in the primary storage of GAGs, and then there's a whole series of cascade events shown in this slide that result in that. But again, the primary storage is GAG accumulation.

Let's talk about clinical now. MPS II or Hunter syndrome, an X-linked recessive disorder, deficiency of iduronate-2-sulfatase. It's an ultra-orphan disorder. I estimate there's only 450 to 500 patients in the U.S. Most geneticists see very few. About 2/3 of the MPS II population developed cognitive impairment with the onset of symptoms between one and three years of age in the neuronopathic form. The attenuator or non-neuronopathic tend to develop later. Premature mortality. Patients die very young. Occurs in the neuronopathic form secondary to the overwhelming neurological progression. They die of brain disease. Although intravenous enzyme is available, there's a high unmet need for treating the brain disease in this population.

Like all inborn heirs, there's a spectrum of clinical activity. In MPS, we talk about neuronopathic too and non-neuronopathic. You can see the dramatic difference in these individuals. Neuronopathic patient died at age 16 or 17, said a few words in his lifetime, never was toilet trained and had a progressive decline after three to four years of age. Neuronopathic MPS II and also the same thing

applies to Sanfilippo, it's a devastating disorder from a neurological and somatic with onset of disease one to three years of age and start a regression at three to six and then they keep declining until they die. The common seen features that impact the quality of life, severe behavior problems including aggression, hyperactivity, and obstinance. They have seizures, communicating hydrocephalus, and hearing loss. There is no treatment for the brain disease for this devastating disorder.

Sanfilippo syndrome. Very similar in terms of the neurologic features but does not have the physical disease we see in the MPS II patients. Four enzyme deficiencies but all have the same relative clinical features characterized by childhood onset, progressive neurocognitive deterioration. There's different forms that you see rapidly or severe or slowly attenuate progression phenotypes. There is now an adult onset phenotype with mild cognitive or even non-neuronopathic form which have been identified. The major clinical manifestations include mental deterioration, hyperactivity, behavior problems, relatively mild somatic features, and death typically in the teenage years of overwhelming disease.

Historically, it's classified in terms of... Divided into three disease phases, that first phase, second phase, and third phase, which I won't talk about, but you can see right there. It's really a progressive disorder. In general, all the classical MPS III individuals follow the same disease course, a progressing phenotype with variable rates of progression. Delayed diagnosis is really common in all the MPS III patients, particularly in the Sanfilippos, which is slowly progressive. Again, there's no treatment for the brain disease in this devastating disorder.

Some of the children shown here all are now deceased. MPS I, another severe form of neuronopathic deficiency of alpha-iduronidase. Onset of symptoms six months of age in the severe form of Hurler syndrome. Again, it's a rare autosomal receptor disorder. Transplantation is the treatment of choice for Hurler syndrome, but you have to be less than two years age. If you're older than two and have some developmental delay, you do not benefit from transplant because the irreversible brain disease. It's important to be aware. We have this example of irreversible brain disease that occurs in all the MPSs, but MPS I is a good example in terms of transplant.

Severity of symptoms. Hurler profound neurological impairment to an adult with Scheie syndrome who is intellectually intact

MPS VII. Deficiency of the enzyme beta-glucuronidase. Somatic in the CNS involvement is similar but can be more severe than MPS I. Non-immune hydrops fetalis is a common presentation in North America for this autosomal recessive. Again, no treatment for the brain disease.

In fibroblasts, we could treat these disorders for 50 plus years. Cultured cells release small amounts of lysosomal enzyme, which my mentor, Liz Neufeld, referred to as correction factors. There's an efficient mannose-6-phosphate

receptor-mediated uptake that occurs in fibroblasts and presumably in most cells, but more importantly, we only need a small amount of residual enzyme in the cell to correct the GAG storage, one or two percent.

What are our current treatment options? Hematopoietic stem cell transplantation primarily for MPS I and IV enzyme replacement therapy. This shows here my view of the world in terms of treatment options. Again for MPS I, the Hurler patients, the treatment of choice is hematopoietic stem cell transplant if you're still intellectually intact and younger, less than two or two and a half. There's really no obvious treatment for any of the Sanfilippo. Transplantation hasn't worked typically because they're diagnose much later and transplantation doesn't work when you have delayed development and irreversible brain disease. You can see here there's five different somatic enzymes approved. I've been heavily involved in both MPS I and II over my career.

Heterogeneity is one of the challenges. The FDA said, "Oh, they can't interpret these." But it's important to realize that the course of disease in a neuronopathic individual occurs, but it's different, but they all will develop CNS disease. So even though they may look a little different early on, they're all going to develop overwhelming disease and die prematurely. It's important to realize that because of the slow and variable disease course, it may take four to six years to observe the result of a successful intervention. These do not happen overnight.

You'll see that slide on the left again a couple of times. This presentation just showing the natural history of MPS IIIA patients showing both a rapid progressing and slow progressing, basically different severities of the disease. But on the right, you see the heparan sulfate. All these patients had elevated heparan sulfate. It's important to note that the upper end of the normal limit is 0.5 or 0.6, and all these patients had greater than two. So these are all CSF heparan sulfate storage and all the Sanfilippos, even the so-called slow progressors.

Clinically diagnosed MPS patients with developmental delays and cognitive impairment already have significant impairment of neuronal function at the time of diagnosis and they're typically irreversible. These are not reversible disorders, so once they have impairment, that's what you're left with. Replacement of the missing MPS enzyme in the brain of a neuronopathic individual with cognitive impairment will not result in improvement of cognitive impairment, but it best will stabilize the patient. Placebo controlled clinical trials of greater than one or two years for a progressive neuronopathic disorder like the MPS to me are unethical. You're basically sacrificing children's brains to show that you have benefit. There has to be a better way and clearly, utilizing CSF heparan sulfate as a biomarker and accelerated approval pathway is the logical solution with long-term follow-up where we can really show the difference.

Why CSF heparan sulfate should be a biomarker for accelerated approval. It's pretty clear we don't need to qualify this biomarker. It's already qualified. Why some enzymes are only active within the acidic pH of the lysosome, heparan sulfate is the primary substrate that accumulates in neuronopathic MPS individuals. CSF heparan sulfate levels correlate with brain tissue sulfate heparan sulfate in animal models. You'll hear from Matthew Ellinwood, from Patty Dixon showing that exact issue. The only way for CSF heparan sulfate to be decreased is the enzyme has to enter a brain has to enter a cell and reduce the heparan sulfate and that cell now will release less of that product. So you have to realize these enzymes have to get into cells to be able to decrease CSF GAGs. Lowering CSF heparan sulfate is reasonable likely to predict clinical benefit. It's probably more than reasonably likely. It will predict clinical benefit if it's done early and prior to significant disease onset.

I just briefly want to show two different clinical trials about some of the challenges. I'm involved heavily in an MPS II phase two three intrathecal trial. Patients were all on weekly enzyme. We did 10 milligrams of intrathecal injections monthly through an intrathecal drug delivery device or lumbar puncture. The first year data demonstrates safety, and that's really the importance. Safety needs to happen for all these before anything else. But once you have safety, then you need to look at other issues. It did meet its pre-specified primary secondary endpoints. Although from my perspective, the younger patients appeared to have significant clinical benefit. The company Takeda is no longer seeking regulatory approval, but still available to allow us to dose these patients monthly.

I just want to show this here. The intent to treat population on the upper there shows a p-value of .053. It didn't meet a specified end point, but if you look now at a post-hoc analysis of younger patients, they clearly had a very significant p-value. On the left shows the CSF GAGs measured at the time using a thrombin assay, a nonspecific assay for GAGs. We got fantastic reduction of GAGs, but in retrospect, the heparan sulfate did not get reduced anywhere near as much, and this is one of the examples the FDA has sort of occluded. Biomarker reduction is not correlated with clinical benefit here. They had a great biomarker reduction initially with the CSF GAGs, but as nonspecific primary [inaudible 00:53:39] and we didn't see a lot of clinical benefit, and they're tossing this back at company saying, "This assay was very different back then."

It turns out neurofilament light chain analysis in the phase II/III study really shows some improvement but modest improvement. But more importantly, there are patients who had elevated neurofilament light change with the higher the level, the less responsive they were, and that's important. You're already pre-existing brain damage. It just supports that dramatically.

I want in the next five, talk about the Denali phase I/II study, which I've been heavily involved with. DNL310 is a protein, is a product that has the antibody fragment against the human transferrin receptor fused to the iduronate-2-sulfatase enzyme of the treatment for brain disease. Forty five patients have

been dosed anywhere from three milligrams to 30 per kilo. It's safe, it's been well tolerated, but all patients have been on idursulfase prior to starting.

You see dramatic reduction of CSF GAGs in the phase I/II study. This is not reduction. This is normalization. Normalization in addition to sustained over time, even with patients with high pre-existing antibody. Based on what I know about this disorder, this data alone should allow accelerated approval to occur because, again, there's just so overwhelming evidence. But then if you look at secondary markers like GM2, GM3, they go down. If we look at neurofilament light chain reduction, you see that go down. So we have additional evidence to support that this biomarker really should be used for accelerated approval.

So my last two slides... Susan, two slides left.

Susan Winckler: Yes.

Dr. Joseph Muenzer: I want to give you my perspective of why the biomarker CSF heparan sulfate using the accelerated approval pathway should be utilized for neuronopathic MPS disorders. These are ultra-rare disorders. It's incredible low prevalence. The biochemistry is really well understood. These are single enzyme defects, the primary event is a defect in GAG metabolism resulting in intralysosomal accumulation due to deficient enzyme activity. That's what happens in these patients. Well understood for 30, 40, 50 years. CSF heparan sulfate is always elevated in the MPS neuronopathic patient.

More important today, and you'll hear from Dr. Fuller in this next session, CSF heparan sulfate can be reliably measured using mass spectrometry. We don't need to qualify this. This is now the technology, where 10 years ago, it wasn't available. We didn't have [inaudible 00:56:23]. The science has advanced tremendously. CSF heparan sulfate can be readily measured using mass spectrometry. The levels for CSF heparan sulfate correlate with brain tissue and you'll see that again from Dr. Ellinwood and Dr. Dixon showing that correlation. It's absolutely there.

Reduction of CSF heparan sulfate reflects brain tissue reduction. The reduction in secondary disease activity, markers of lysosomal dysfunction, GM2, GM3 and neural injury support the relevance. I guess that's my time to stop soon. I have two more primary markers.

Susan Winckler: You've got two [inaudible] now.

Dr. Joseph Muenzer: Reliance on clinical efficacy with placebo-controlled trials demonstrating effectiveness is unethical in this population. These are progressive irreversible diseases. If this was a reversible disease, then I could find... But either not reversible disease. Once you have damage, you have damage. And again, regulatory flexibility is needed to bring treatment to individuals with

neuronopathic MPS using the FDA's 2020 industry guidelines. The guidelines already there to do this. Thank you for your attention.

Susan Winckler: Thank you, Dr. Muenzer. That was really helpful, particularly for the many people we know in the audience who are not as familiar with MPS and the topic to help us get into the case study. We will see you back after the next two presentations and we'll do a joint Q&A. So let me now introduce Mark Dant, who is the founder and volunteer executive director of the Ryan Foundation. I would say, Mark, it's always helpful to remind us of the patient at the center of our discussions. Can you pick that up and provide that for us?

Mark Dant: I can.

Susan Winckler: All right. Thank you, Mark.

Mark Dant: First I want to thank you, Dr. Marks, for speaking this morning and helping us all understand our system and thank you, Dr. Muenzer, as well. You've just heard from the leading expert in MPS in the world. And throughout the day, you're going to hear from doctors and scientists who are the top peer-reviewed experts in MPS globally. I am neither. After 32 years of service. I'm a retired assistant chief of police from the Dallas area, but more importantly, I'm Ryan's dad. This photo was taken of Ryan at age three in 1991, not long after he was diagnosed with MPS I. When Ryan was diagnosed, science could not tell us whether he was severe or attenuated. Only time and watching his progression would do so. Thirty three years have gone by now. There was no FDA approved treatment for any of the MPS disorders back then. We were told Ryan would suffer and die within 10 to 15 years.

For the next few years I spent every off-duty hour in the public library trying to learn everything I could about MPS, most of it of which was written by Dr. Joseph Muenzer. The more I read, the clearer it became that our answer would be coming from the partnership of the patient, the scientist, the physician, industry, and our FDA. In December of '97, an enzyme replacement therapy trial for MPS I began. Ryan enrolled, and on February 13th, 1998, received his first infusion of the drug that would put back in his system what he was not able to make himself, clearing the stored substrate that, by the age of 10, was already destroying his body. Ten patients enrolled in that trial. The New England Journal of Medicine would later publish that all 10 patients improved. The initial review team at the FDA suggested that the sponsor bring the data forward for a review without a double-blind placebo control arm, but before the sponsor could do so, the lead of that FDA team was diagnosed with brain cancer and passed away.

The application was reassigned to a second team of reviewers who called for a double-blind placebo control arm, delaying the eventual approval four years, and frankly nearly bankrupting the sponsor. The second trial gathered the same data as the first. This time all 44 patients improved, and just like the first trial, the drug lowered the stored substrate in their bodies. The double-blind placebo control arm taught us, the patient community, something quite profound. We

were taught that some of our children would have to suffer through and sustain irreversible damage in order to satisfy regulatory requirements. In one case, two sisters enrolled with the younger sister receiving the drug while the older was placed in the control arm. Instead of receiving the drug for six months, she sat through weekly four-hour IV infusions of salt water. Within a few weeks, the younger sister who was receiving the drug began pushing her older sister into the hospital every week for their infusions. Who was receiving drug and who wasn't became painfully clear to everyone, including the two girls themselves.

Since that first MPS I trial 21 years ago, MPS II, IV, VI, and VII all have an approved ERT to treat somatic disease. All of these approvals showed substrate reduction, yet all were forced to have a control arm in the trial. Despite two decades of data regarding the potential use of substrate reduction as a primary disease activity biomarker in MPS disease, our FDA has yet to accept the long-term reality that the downstream effect of lowering substrate accumulation in all of these disorders has improved not only the quality of life but extended life itself.

By law, we have one FDA, one set of standards. For approval, drugs must be proven to be safe and efficacious. No other hidden regulations. Safe and effective. Let's compare neurocognitive MPS conditions and pediatric cancers. Approved treatments for cancers often do not cure the cancer, yet they do extend the life from years to sometimes only months. Extended life is considered a win. Parents of children with cancer are willing to allow physicians to poison their child's immune system and then, in some cases, give them a drug that may only have a 20% chance of stalling the disease progression and extending life because they know it is the only chance for their child's survival. Yet, in ultra-rare neurocognitive conditions like these MPS disorders, slowing progression and extending life is not enough to gain approval.

Why the difference? Pediatric cancer drugs are approved with no control arms because we understand that it is simply unethical to watch a child's cancer progress, all for the sake of data. Yet with ultra-rare conditions affecting the brain, like MPS I, II, and III, we are told we must accept that if our child is displaced in a control arm for as long as two years, we will watch them decline, lose skills, and suffer irreversible brain damage before finally being moved to the treatment arm. Is that not unethical as well?

Meet Cole, who, at the time of this video was taken, was two and a half and has the severe form of MPS II. Start the video, please. Oh, let's go back. Start the video.

Video: (singing).

Mark Dant: Cole no longer sings or talks. He has lost that ability. At five, Cole's singing stopped. At seven, his words went away until he spoke only two words, help and mommy. At nine, Cole spoke his last word, "Mommy." Cole has not spoken since. He's almost 14. It's been five years now since Cole's mom heard his sweet

voice. Cole entered that intrathecal enzyme replacement trial that Dr. Muenzer spoke of when he was four years old. Each month for the past 119 months, Cole has been sedated and received the enzyme he's missing directly into his cerebral spinal fluid through a lumbar puncture. Though Cole no longer speaks, at almost 14, he can do things I have never seen severe MPS II boys do in 30 years. By 14, most, if not all severe Hunter boys are no longer walking or communicating. Yet Cole often goes on three-mile walks with his mom, completes 50-piece jigsaw puzzles, and runs to his school bus every morning. Play video, please.

The dose given in that intrathecal trial was widely believed to have been inadequate. Yet the heparan sulfate levels in Cole's CSF were lowered each month for over 10 years because the enzyme he can't make himself was replaced, and at least some of the stored substrate in his brain was reduced. But because lowering a primary disease activity biomarker, like heparan sulfate, was not considered an acceptable trial endpoint, participants in the trial were subjected to taking the same two-hour standardized cognitive test every six months for 10 years. Cole, after years of taking these same tests every six months, refused to take it any longer. He'd do anything to get out of it. He would fake falling asleep. He would crawl onto the table. One time, he even picked up his chair and turned it around and faced the opposite direction of the neuropsychologist.

Unfortunately, because Cole was born with it and he wasn't the only one, his refusal resulted in Cole's scoring counting as zero. And Cole wasn't the only one in the trial that refused to take it. So the sponsor, after 10 years of compiling data and not being able to quantify clinical improvement, discontinued the trial. Had heparan sulfate been accepted as a primary disease activity biomarker by the FDA, objective data measurements could have been used to reasonably predict the clinical benefit we just saw Cole is experiencing. MPS disease does not improve on its own. It is scientifically and medically impossible. The reduction of the storage substrate took Cole off the normal timeline of this progressive brain disease. You just saw that in the video.

Now, let's meet Sadie. Sadie has MPS IIIA. At two, Sadie was cognitively ahead of her neurotypical peers and could say her ABCs, count to 10, and spell her name. At three, Sadie enrolled in a blood-brain barrier crossing ERT trial. After only a few weeks, her stutter went away. A month later, before any results were available, the sponsor announced a reorganization to focus on later-stage drug development and a plan to divest the Sanfilippo treatment. But parents were allowed to continue the drug for the two-year duration of the clinical trial. The sponsor has sought out other companies to acquire the promising treatment, but to date, all have declined citing a lack of confidence in the regulatory flexibility needed to gain approval.

Sadie did well on the drug, an advance from the Bayley to the Kaufman. At the end of treatment, Sadie knew hundreds of words and spoke in multiple-word sentences. She was very observant, witty, and loved to sing and dance. Results

showed that the drug passed the blood-brain barrier in all patients. Heparan sulfate levels were lowered by 70 to 80% and correlated with stabilized cognitive scores on the Bayley scales. For several months after the trial's completion, Sadie remained stable, but as heparan sulfate started to build back up, she started to regress. Over the last year, Sadie has lost nearly all of her once large vocabulary. She can no longer tell her family that she wants her favorite food, ice cream, or that she wants to watch her favorite show, Peppa Pig. Sadie is struggling more with eating and mobility. She gets tired faster, and her parents frequently now use a wheelchair. Play the video, please.

Video: What's this?

S-A-D-

Good job.

... I-E.

And what does this say?

Sadie.

What's your name?

Sadie Rae Haywood. Ask me again, I'll tell you the same. My name is Sadie. My name is Sadie.

What's your name?

Mark Dant: Many believe treatments are too intrusive or too risky simply because they are not acceptable to them personally. Disbelief does not consider the reality of our world, where the alternative to treatment is death. The Cure Sanfilippo Foundation published this important study. The mom's statement about her willingness to take a risk for even six more months of quality with a daughter, Liv, who has MPS IIIB, is universal among parents with children with terminal diseases. Tomorrow, Cole will undergo his 120th lumbar puncture to receive treatment. My son, Ryan, has been receiving off-label quarterly ERT lumbar punctures for more than 10 years. The risk is worth the potential outcome, life. We are no different than the parents of the pediatric cancer patients I spoke of earlier. We want our children to suffer less and live longer. We cannot continue to expect biotech partners, many of them startups, to keep investing in our children's treatment when the current system takes years in clinical trials. Our regulatory system must evolve with the science. The ultra-rare disease ecosystem is not aligned with the regulatory process, and transformational science is being left behind.

Depicted here is the current therapeutic pipeline for the four diseases of MPS III, MPS A, B, C, and D. At one time, 17 companies had MPS III programs. Ten have now dropped their programs, and one has an uncertain future. Many of the parents of children in these clinical trials saw positive life-skill changes. The measure of primary disease activity biomarker that leads to accelerated approval will allow scientists the opportunity to prove these drugs are working for our children. Cole and Sadie are a testament to the failed system of review. While regulatory guidelines are struggling to keep up with the science, our children are dying. In March 2020, the FDA issued guidance for the type and quantity of evidence necessary to support effectiveness for replacement or corrective therapies. Notice this section relevant to our discussion today. When the pathophysiology of a disease is well understood and the mechanism of action of the drug or biologic is well characterized, specific drug-induced substrate reduction in relative tissue or tissues can have a reasonable likelihood of predicting clinical effectiveness.

A clear demonstration in a clinical trial or trials that an exogenously administered enzyme or drug results in substrate reduction, i.e., it reaches the tissue of interest, this can serve as the basis for accelerated approval. "Reasonable likelihood to predict," everything these experts will speak to today will point specifically and precisely to that phrase. We can objectively measure life ability changes through a primary disease activity biomarker that has been proven, over the past 26 years, to have a predictive outcome. And as Cole demonstrates quite well, even a small change in substrate levels over a 10-year period has changed the course of his disease. Over the past 30 years, I have attended countless MPS funerals of children who could no longer wait for our regulatory process to catch up to the science. For Ryan, the partnership of patient, science, physicians, industry, and our FDA found his tomorrows by effectively reducing the stored substrate in his body and stabilizing his brain disease before it was too late.

At 35, he's the longest-treated person in the world with an MPS condition. Graduated college, got married, bought their own home, and now works as a patient advocate in a small biotech firm. The constant evolution of science is giving him the opportunity to live his life and, hopefully, be here like today, when science is improved, and better treatment is here. All children deserve the chance to live. Our FDA has the flexibility and authority to qualify heparan sulfate as a biomarker for MPS brain disease, opening the door to accelerated approval and treatment for so many children that today's science, today's partnerships have the ability to treat. This generation of our children needs our FDA partners to have the courage to do so. As you listen to the speakers today, please remember that behind every statistic and every heparan sulfate level is a child and a parent who thoroughly understands the risk of treatment compared to the certainty of death without it. We do not want your sympathy. We do not need your empathy. We need action from our FDA partners, and we need it now. Thank you.

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

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

Susan Winckler: So we started with the baseline of just getting a better understanding. Again, some of you know this area very, very well, but we have hundreds of people who don't and have just learned quite a bit. And now, we want to move to a topic that Dr. Marks raised this morning, is particularly important, which is the measurement challenge. And so to learn more about measuring glycosaminoglycans like heparan sulfate, we are going to turn to Dr. Maria Fuller, who is winning the award for the longest distance traveled for today, and she had competition. But Dr. Fuller is a clinical scientist specializing in biochemical genetics who leads the National Referral Laboratory within the statewide public pathology service in South Australia. Dr. Fuller, take it away.

Dr. Maria Fuller: Thank you very much. Thank you, Mark, for a very profound talk. It was truly wonderful. And my remit here today is to show you that the science to measure heparan sulfate in the CSF is actually possible. I'd like to also extend my gratitude to the Reagan-Udall Foundation for the opportunity to be here today and participate in this very important symposium. So as I said, my remit is to show you that we can measure glycosaminoglycans and heparan sulfate, and what we can do today as what we couldn't do before. So I thought I'd start by just defining what glycosaminoglycans are for the audience that may not be overly familiar with them. They're essentially sugars. They're carbohydrates. And if you like there, they're carbohydrate chains of proteoglycans. And if this pointer... No, I'm not going to be able to... Sorry.

Speaker 2: I think you point there, right?

Speaker 1: Right here.

Dr. Maria Fuller: On this one? I'll give up because I'll just waste too much of everyone's time. So they are covalently linked to a protein core except for hyaluronic acid, shown at the fourth one there through this tetrasaccharide linker. Importantly, they are repeating disaccharide units. And if we look at heparan sulfate, abbreviated as HS at the top, you can see it's an iduronic acid and an N-acetylglucosamine. And they're the two sugars that make the disaccharide. And these are just essentially repeating disaccharide units. There's four or five main classes. And that really depends on whether you consider dermatan sulfate to be a separate class because it's an isomer of chondroitin sulfate. They have a high degree of heterogeneity. And I'm sorry, something's going to stew with my slides. Some of the words have become jumbled. But essentially, you can see throughout the oligosaccharide chain, the repeating disaccharide units. There's levels of sulfate, and there's levels of acetylation. And they're critically important for the function of GAG.

Now, GAGs are essential for cell function. They're present in all cells. They're ubiquitous, and they have critical roles. They're highly dynamic, which means

they participate in a lot of biochemical processes to keep the body functioning. And you can see some of these sort of processes are depicted in that schematic on the right. And you can see that core protein in white down the middle, which is what I was showing you, that the glycosaminoglycans are covalently attached to. And then there's these sugar chains shown as the blue dots. And you can see them, they're interacting with growth factors. Very important for signal transduction. And as Dr. Muenzer touched on earlier, some of them are profound in particular tissues. And chondroitin sulfate, for example, is really the driving force for ligament and tendons. Dermatan sulfate, it's very important in wound healing. And probably the most profound of all of the glycosaminoglycans is heparan sulfate, and that's because it plays critical roles in cell signaling and transduction to allow cells to communicate with each other and cells to function.

It's also very important in the brain for morphogenesis and development. Now, what else is important is the quantity of glycosaminoglycans. That is also critical. And this slide here just attempts to depict all the metabolic reactions that are going on in our body to keep us functioning. And like all the other proteins, fats, carbohydrates, glycosaminoglycans are also carbohydrates. So they need to be metabolized critically as well. Now, there's a group of inborn errors of metabolism where there's actually a failure to actually metabolize these compounds properly. The net result is either a shortfall in energy production or it's the accumulation, often of material that should normally be metabolized. So I think the glycosaminoglycans should normally be metabolized, and they're not because we have genetic deficiencies in enzymes that are required to turn them over. And I'll talk about that a little bit more in a minute.

But probably the most well-known inborn error of metabolism, whoops, I'm going the wrong way, I'm sorry, is the inborn era of metabolism called phenylketonuria. And you've probably noticed on diet soft drink cans. It actually warns people that this soft drink can contains phenylalanine. If you have phenylketonuria, you cannot metabolize the phenylalanine. The toxic metabolite accumulates in the brain and causes profound neurodegeneration. Obviously, we test for phenylketonuria at birth by measuring the metabolite, not the enzyme, not the genetics. We measure the phenylalanine in the drug blood spot at birth, and treatment is withdrawal of phenylalanine from the diet. So the MPS are no different. They are inborn errors of metabolism, inborn errors of carbohydrate, glycosaminoglycans metabolism, and the net result is that these glycosaminoglycans accumulate. And Dr. Muenzer has already told us the ramifications of that occurring.

Now, if we take a little, tiny portion of the previous slide and we just focus on GAG degradation, and I've just got heparan sulfate, dermatan sulfate, and keratan sulfate shown here as an example. So this is an excerpt of the previous slide that just shows GAG degradation. Now what's critical here is there's no redundancy in the system. These enzymes works sequentially. So let's look at the top heparan sulfate. You can see that the terminal residue has a 2-sulfate. So iduronate-2-sulfatase will remove that 2-sulfate leaving iduronidase, that

then allows iduronidase to remove the iduronic acid. But if the two iduronate-2-sulfatase is missing, iduronidase cannot remove the iduronic acid. So it's sequential, it's stepwise, and there is no redundancy. If there's a metabolic block, it stops there, and that substrate accumulates. Now, here's another way to show it in a freehanded drawing by myself, which is less sophisticated than the previous one, but maybe explains in a little bit more simple terms.

So blue and red are the sugars, repeating those repeating disaccharide units. We've got these endo-hydrolase enzymes, which I will mention later, that cleave the really long sugar chains, if you like, into shorter chains. And then we have these exo-hydrolases, and they are the MPS enzymes. So they remove the non-reducing sugar sequentially stepwise. If in the presence of an NPS disorder, that will stop, and you will get these partially degraded fragments of GAG where the non-reducing end is the substrate for the enzyme deficiency because it can't be further unzipped. And obviously, the net... Oh, my slides have become jumbled. The net result is the accumulation of sub-GAG in the cell. And you can see the normal cell, which, for some reason, is now taking over the whole slide. And the MPS cell, you can see these white vacuoles, which are the lysosomes, and they are full of incompletely degraded GAG. And that leads to the chronic, progressive deteriorations of cells, tissues, and organs and the clinical phenotypes which both Dr. Muenzer and Mark have described earlier.

So why and how have GAGs been measured? So GAG have been measured longer than I've been on this planet. And it started with simple spot tests. The Berry spot test, I think, was the first one. And then, the urine dye-binding, often referred to as the DMB GAG measurement, has really been the mainstay of glycosaminoglycans measurements. And that's a picture there in our lab of the GAG assay. It exploits the ability of the charges, the ionic properties of the GAG, to bind with dyes and form a color complex, which can be very easily measured spectroscopically. And you can see the different colors in blue across those samples there. And if we go back to Dr. Marks' sort of criteria for a validated biomarker, this sort of urine GAG method probably fails on every single one. It has poor precision. For those of you who have done it, it is a pain in the neck. To get the same answer, you have to often do it multiple times, you have to do multiple dilutions. The sensitivity is woeful. You have patients that will have elevated levels, normal levels may or may not have the disease.

The biggest problem with it, it's a total measurement. So it's not actually specifically measuring the fragments that are reflective of the substrate. So MPS IIIA, for example, only stores heparan sulfate. It doesn't store dermatan sulfate or chondroitin sulfate. All the enzymes are there to unzip those GAGs. But this doesn't differentiate. It measures total GAG. The other problem is that concentrations vary with age. So as children are growing, GAG concentrations fluctuate, and it's certainly not diagnostic. So what tends to happen is if you get an elevated DMB GAG result like this, you have to do even worse laboratory methods, which is this awful MPS electrophoresis onset of those acetate. And again, for those of you have done these, these are horrible. Again, they're not diagnostic, but they will give you a pattern suggestive of a particular MPS

disorder, which warrants further investigation with molecular testing to identify the molecular variants or the enzyme activity to show that that's reduced.

Now, I could go on for another half an hour about all the problems with these in the diagnostic setting, but I'm not going to. I'm just going to share with you one case presentation to put a real-life example to the problem with measuring GAG. So our lab received urine from two siblings with a strong clinical suspicion of mucopolysaccharidosis. As everyone does, the first-tier testing was a sort of general urine metabolic screen, and we measured the GAG. In Sibling 1, you can see it was slightly elevated, and Sibling 2, it was normal. We then went ahead and measured the type of GAG in both patients using a high-resolution electrophoresis, and they were both normal. These poor siblings spent the next five years with a broad sort of diagnosis of a nonspecific skeletal dysplasia until a very astute clinician said, "I'm sorry, these siblings have MPS until proven otherwise." And had we had a specific oligosaccharide marker for the disease, we would've been able to diagnose these two siblings the first time they came to us.

And you can see Sibling 1 and Sibling 2 with this oligosaccharide, which I'll talk about in a minute. Both of them have elevated levels that is clearly above normal. And what was this oligosaccharide? If we come back to this GAG degradation pathway, which you're probably going to get sick of me showing today, you can see that in the red circle there. That was what we measured by mass spectrometry. And that is the substrate for the enzyme that's deficient in MPS IV. So there was a terminal galactose 6-sulfate, and we could measure that disaccharide by mass spectrometry.

Now, I'm just going to give you a little bit of an introduction, and again, sorry, these slides have become chopped, about mass spectrometry. It's really been a game-changing tool as far as analytical measurements are in the biochemistry laboratory. And to be perfectly frank, it's just a hideously expensive weighing machine that can measure things in the femtomole range. So really, really small concentrations. Not only can it do that, it's improve sensitivity and specificity. We actually know exactly what we're measuring. If we put internal standards in, and an internal standard is just a concentration of a known substance that is isomerically similar to the analyte that we want to measure, the quantification is astoundingly good.

We're actually measuring proper concentration units. So our labs got rid of dye-binding for total GAG and electrophoresis eight years ago, and we've replaced it with this. We developed a method based on this idea that GAG fragments, the substrate being the deficiency of the enzyme, is unique for every single MPS. So sticking with urine, just use a very small amount of urine. We had a dramatizing agent, we had an internal standard, and we can pretty much identify every single MPS in the urine quantitatively. I'm not going to go through all the criteria for validation. Dr. Marks has already mentioned those. Why this won't stay on that slide there? I just have to hold the finger down. Are you able to just... It

seems to have a mind of its own, and it's clicked to the next slide. Would you mind just hold-

Speaker 2: Yeah, if you say next slide, I think, or do you need him to hold it?

Dr. Maria Fuller: That slide there, are you able to just leave that on the screen for-

Speaker 2: It does have a mind of its own.

Dr. Maria Fuller: I promise, I'm not touching it. I'm not going to speak to the criteria for sensitivity, specificity other than to say that in Australia we have this NPAAC guidelines, which validate to international pathology standards, which cover all the criteria of robustness, sensitivity, accuracy, precision, for us to be able to get it validated into the diagnostic service. So before we could offer it as a test... So our lab in Australia provides diagnostic services for all of the patients in Australia and New Zealand with lysosomal disorders. And we didn't want to do the DMB GAG anymore. We replaced it with this. So we had to get it validated. We introduced it into our diagnostic service in 2016, where we had it accredited by our national accreditation body, which is no different to CLEAR, CAP, and the UKAS society.

In the last eight years, we've identified 55 positives, and there's a list. You're not going to be able to read what they are with this slide continuing to go forward. But the advantage of that is I can gloss over the fact that we did have two false positives, and you don't need to see that because what I need to say about those two false positives is they were laboratory errors. They were nothing to do with the method. They were us misinterpreting peaks early on in the space. And so we really can say we did it properly, and we would have zero false positives. And a testament to that is this ERNDIM, which is an external quality control program. So Dr. Marks touched on that earlier about all laboratories that participate in this. Sent samples from the one reference laboratory. They all have to test them blindly and send the results back. And that's a way of verification that we're all using the methods appropriately and getting the right answer.

And I can safely say to you that we've had, over the last eight years, perfect external quality control, and it won't stay on long enough for me to convince you of that. Okay. So enzyme replacement therapy. Dr. Muenzer has touched on enzyme replacement therapy. This just shows that using the same approach, we've got 12 children here on treatment, and you can see that there's a precipitous drop in the concentration in the urine of this disaccharide with treatment. The filled circles showing the baseline before the patients went on to treatment. So just that GAG, it can be used, and GAG really was the mainstay of the enzyme replacement therapy trials. You can measure this oligosaccharide. Now, again, Dr. Marks touched on this, at the time in 2016, we were probably the only lab in the world doing this. And I shared a methodology with Dr. Michael Gelb, who many of you know has been a pioneer in the newborn screening space.

And he said, "Maria, I'm not interested in urine, but I'd love to see if it works for newborn screening." So we shared the methodology. It was good cross-validation to have two labs on either sides of the globe, and he set it up for newborn screening. And you can see, it's actually fantastic for newborn screening. You can see the patients on the right here, those with the Scheie phenotype and the severe phenotype all have elevated levels of the signature disaccharide. And importantly, patients with the pseudodeficiency and carriers, which we all know are giving false positives in the newborn screening, are the same as the controls. MPS II, just more recently added onto the RUSP. Again, the same is true. The pseudodeficiency in the same concentration as the newborn controls, with the MPS II patients showing elevated levels. And more recently, Michael has published this that it works for all of the mucopolysaccharidoses as a second-tier test following a reduction in enzyme replacement therapy to rule out any false positive. So really quite profound in the newborn screening space. Now, I'm not going to talk too much about the methods, but there are essentially two different methods. You can either measure the native non-reducing end that I explained to you is generated by the deficiency in the enzyme that is the specific enzyme substrate, or you can depolymerise the GAG polymers into disaccharides using those endohydrolase enzymes. You can do it chemically as well. But a couple of the disadvantages of doing it chemically are that the structural detail is lost. And there have been some reports of contaminating peaks in the mass spectra that are a little bit hard to identify. So the majority of people do it by enzymatic depolymerisation, and the advantage of this is it increases the concentration of the analyte for the mass spectrometry, because you're generating multiple disaccharides rather than just measuring the native one that's on the non-reducing ends.

One of the disadvantages with mass spectrometry is its size. So you can't put large ... It has a mass range. So, even though it's only an expensive weighing machine, you can't put big things in, because it's outside the dynamic range. So, this is a way of chopping up these into smaller disaccharides that can be measured. And rather than going through all the details of validation of methods, because you will go to sleep, what I want to show you is that if you validate methods properly, they correlate.

And this was done ... There's a bit of a backstory about us living in Australia and not having access to enzymes about why this happened, but this is actually 12 patients who were tested with our method in Australia and ARUP here in the US completely blindly, because we couldn't get access to the enzyme during COVID to be able to validate the enzyme method. And it's like, "Well, we have a method that works just as well, and here it shows absolute correlation. So I think if we're going to get into arguments of which method's better, show the methods are validated, and they'll correlate, and give you the same result. And they kind of have to, because we know exactly what we're measuring by mass spectrometry. We're not measuring total GAG anymore.

So in the last few minutes, I hope I've got left, I just want to do a bit of a deep dive into heparan sulfate, because I think heparan sulfate is the most critical

GAG as far as cell function, and it's also the one that's the driving force in pathology for the neuropathic MPS disorders, which we are really here to discuss today. So again, I've got the heparan sulfate degradation pathway here. It's composed of uronic acid and acetylglucosamine. And the uronic acid can either be iduronic or glucuronic acid.

We have these sulfated domains and these acetylated domains, and we tend to talk about them as NS and NA domains, and they're really critically important for function. So, there's been a lot of work done with specific structures of heparan sulfate about how they bind to growth factors and signaling properties, and these are critical for neuronal development and function. So, you can see on the right here, the MPS shown in purple, where there's a specific block in the unzipping of heparan sulfate. And what's also important in these MPS disorders is that heparan sulfate is present at birth. We know that because we can measure it in dried blood spots in the newborn screening program, so we know it's present at birth. We also know that it's present at birth because of all the work done in animal models when treatment has been trying to start prophylactically. And it's present at birth in the brain.

So, I've talked a lot about urine. So, let's have a look at some other urine and blood spots. I've shown you hopefully quite compelling data that it works. Let's look at some other readily accessible samples. So if we look at plasma at the top here, we can see that there is none of the disaccharide in the control plasma samples, but you can see the specificity. We've got MPS-IIIA in red and MPS-IIIB in blue. But you can see that there is no crossover. If you measure the disaccharide unique for MPS-IIIA, in that first graph on the left, you can see there's nothing in the IIIB's. So the IIIB's are the same as the controls, because it's specific for MPS-IIIA and the converse is true. And you can see in CSF as well, exactly the same thing is borne out. The marker is specific for MPS-IIIA and not IIIB, because we are measuring the substrate for the enzyme deficiency. We're not measuring total GAG.

Heparan sulfate in the brain is reflected in the CSF, and I think this is critically important. We're using CSF as a surrogate, because we're not going to be taking brain biopsies. But you can see that the brain and the CSF, this is Pabinafusp, which was licensed in Japan in March, 2021. And you can see there is a mirror image. We've got the wild type where there's no heparan sulfate at all. We've got the knockout, which is the untreated mouse model, and then we've got three different doses of the enzyme. But you can see the CSF is a mirror of the brain. So the CSF does reflect what's going on in the brain. We've already seen the slide before from Dr. Munzer, but again, if you introduce a therapeutic intervention, you can see that the concentrations of heparan sulfate in the CSF reduce dramatically. So again, it is a biomarker of therapeutic intervention.

So heparan sulfate oligosaccharides are biomarkers, and I think when we think of biomarkers, I think on PubMed, I Googled biomarker just for something to do on the long flight over, and I think there's 1.5 million articles in the literature. But if you do, "Biomarker and MPS," there's 335. Now considering it's a rare

disorder, that's quite a lot. And biomarkers take many forms. Blood pressure is a biomarker, phenylalanine is a biomarker for phenylketonuria, cholesterol is a biomarker for cardiovascular disease, and heparan sulfate is a biomarker for MPS. We can see that it meets the criteria. It's a characteristic that is objectively measured. It's not a subjective measurement. It's objectively measured in a laboratory under quality control in a sample that you have no idea whether it's an MPS or not. It is an indicator of a normal biological process, because it's not there if you don't have the disease. It is evidence of a pathogenic process because it is there if you do have disease. And it also shows that it reduces with therapeutic intervention. So it ticks all the boxes as a biomarker for the disease.

And just before my summarizing slide, I'm going to show you another case presentation, because a lot of people say, "Oh, why aren't you measuring the gene? Why aren't you measuring the enzyme?" The gene and the enzyme are problematic. They are the mainstay of diagnosis, but they form the second and third stage of diagnosis. And I'm just going to show you a case example that exemplifies the power of the heparan sulfate biomarkers and the problems with enzyme activity and molecular. And the slide's doing its own thing again.

So we received amniotic fluid in our lab to test in a fetus at 31 weeks gestation because of a very large liver. And as everyone is on the genomic revolution at the moment and using next generation sequencing, we did exactly the same thing and we measured 151 genes that could explain the hydrops, the large liver, and of course lysosomal diseases, because they have a phenotype that's sometimes hard to predict. And we identified a variant, a hemizygous variant in the iduronate-2-sulfatase gene. Now it causes MPS-II, this gene. This variant was predicted according to the ACMG guidelines to be pathogenic. It was a base from an adenosine to a [inaudible] gene, a changing an aspartic acid to a glycine. I'm having trouble with these slides. Quite an important physiochemical difference, so it was predicted to be pathogenic.

So at this stage we were telling the parents that they had a baby with MPS-II, but what did we do? What do good biochemical geneticists do? We measured the enzyme in the amniocytes and it was significantly reduced. You can see it's just 10% of the normal range. So again, the parents are being prepared that they've got a carrying an MPS-II baby. But then we measured the signature oligosaccharide and there was nothing there in the amniotic fluid. The baby was born. He was unremarkable at birth, completely normal, and he had no signature oligosaccharide in his urine and/or his blood, which I'm sorry is missing from the slide. It turned out he had an older brother who was eight at the time of his birth. He had exactly the same genotype. He also carried this variant. He was phenotypically normal and he had no signature oligosaccharide in the urine. It's now three ... It's actually nearly four years since the boy's birth. Neither of them have any signs of MPS-II and both are doing very well, but their mother actually carries this variant extinct, hence why the boys have it.

So, this is just an example. Relying on genetics and relying on enzyme activity is problematic. So, I'm about to finish now just with a few final statements. I think

the important thing is we are no longer in an era where we're measuring GAG. We have mass spectrometry everywhere. Now, people are talking about mass spectrometry measuring everything in a diagnostic service lab. We're measuring specific oligosaccharides that are disease specific. They tick all the boxes to be a reliable biomarker of disease activity. The heparan sulfate oligosaccharides measured in the brain are reflected in the CSF, so we can use the CSF as a fluid. They are disease specific, so we're not going to have any confusions about the different diseases. Unlike GAG, they're highly precise, highly sensitive, and as long as the people in the laboratory are doing the right thing, you will not have any false positives. If you validate the methods according to the NPAC, you go through sensitivity, specificity, robustness, accuracy, they will correlate. And these are what caused pathology.

Dr. Muenzer put up a beautiful slide earlier that showed the accumulation of GAG and listing a whole cascade of biological processes. The enzyme doesn't cause the disease. The gene doesn't cause the disease. This is what causes ... This is the driver of pathology. And I'm going to go ... And I've said, "Not the enzyme," because I think one of the other problems with the enzyme is we don't really have a good handle on how much is enough. And I know when people are talking about clinical trials, they say, "Oh, it's 10% of normal." What's 10% of normal, when you've got a reference range of 90 to 140? Is it 10% of 90? Is it 10% of 140? And the case example shows the problem with measuring enzyme activity. We do not know how much is enough. And I'm going to go one step further and say it's highly likely to translate to clinical outcomes. And I know you're going to hear more about the clinical efficiency later on this afternoon. And I think I will finish there and say thank you very much, especially with all my slide problems.

Q&A Session with Morning Case Study Presenters

Mark Dant, Founder and Volunteer Executive Director, Ryan Foundation
Maria Fuller, PhD, Professor, Genetics and Molecular Pathology, University of Adelaide
Joseph Muenzer, MD, PhD, Professor, Pediatric Genetics and Metabolism, Univ. of North Carolina at Chapel Hill

Susan Winckler: So, I do have a couple of questions that came in already on the Zoom and by the cards. So, I'm going to jump into those and I'll invite folks to continue to submit them. So this one, Dr. Muenzer, is probably for you, but I'll invite anyone to jump in. And you also have the benefit if you want to say somebody's going to talk about that later, you may defer questions to later sessions. They don't have that benefit, but you do. So here, the first question, heparan sulfate has been described to cluster certain cytokines to drive inflammatory signaling in some settings. Is that considered relevant in MPS pathogenesis?

Dr. Joseph Muenzer: It's relevant, but it's really a secondary event. Again, the primary storage is GAGs, and there's all sorts of ... I'm not sure my mic's on.

Susan Winckler: Yep, mine? Okay, there you go. See? Resolved.

Dr. Joseph Muenzer: Again, the secondary event, a lot of them occur. One of those is inflammation driven by the toxic effect of the GAG. But again, whatever happens, there's a lot to learn yet of basic sign. But we know that the GAG storage causes all sorts of clinical disease. So again, it's one of those things. We don't need to know exactly how it's caused. We will with time. We're learning much more, but ...

Susan Winckler: Mm-hmm, so a point of further exploration, but of less-

Dr. Joseph Muenzer: It's not needed to validate heparan sulfate as a biomarker. That's the key point. We already know it causes significant disease itself.

Susan Winckler: Okay, helpful. So, you each mentioned the need to treat early. So is there an optimal age to select patients for treatment? It's as early as possible. I'm reflecting a question that came in.

Dr. Joseph Muenzer: I'll start. Certainly treating as early as possible is ideal. But again, for most of these disorders, we don't have early intervention for MPS-II. Only a few states are doing newborn screening. And for San Filippo, there is no screening method. So again, we're waiting for the clinical diagnosis. Optimal treating is one thing. In terms of how we do those treatments is also the challenge.

Susan Winckler: Mm-hmm, okay.

Mark Dant: I would want to add one quick point to that. The average age of diagnosis for MPS-III is about four and a half to five years of age. There is no newborn screening. There's a catch-22 there. In the US, we all know you can't have newborn screening unless there's an approved treatment. How do you get an approved treatment if you can't find the patients before the age of four and a half or five, if we only treat the early? We need to treat the patients with the disease. We do that and we will have better outcomes.

Dr. Joseph Muenzer: One of the challenges of treating early is these patients may look good. They may continue their normal course. Remember, the FDA currently wants clinical benefit. If there's no change of that, clinical benefit absolutely is clinical benefit, but they don't deteriorate.

Susan Winckler: Mm-hmm, mm-hmm. So now, I have a measurement-specific question, and you addressed this a bit, Dr. Fuller, but I'll ask it to underscore it. If the GAG measurement can be done in plasma, why not focus on plasma rather than CSF? And you answered this in some of your presentation, but ...

Dr. Maria Fuller: Yeah, I mean it can be done in plasma, it can be done in urine, and it can be done in blood spots. And I'm not advocating that that's not done, but I think if you want an actual measure of what's going on in the brain, then that's the CSF. I think we have evidence that what's occurring in plasma, blood, and urine does reflect what's in the brain if it's a neuropathic disorder. But I think we have direct evidence and mirror, if you like, of what the CSF in the brain looks like.

Dr. Joseph Muenzer: I just want to add to that, that the GAGs are highly charged molecules. They do not cross the blood-brain barrier. And so, to measure something in the plasma or the serum looking at brain disease is totally irrelevant. It should not be used. We need to look at the CSF heparan sulfate in the spinal fluid and that really reflects brain tissue as you'll hear in future speakers. And that's a dynamic, CSF fluid turns over. When it goes down, that reflects impact of intervention, as I showed you in an ALI study. They're lowering brain tissue GAGs and helping those brain cells.

Susan Winckler: Mm-hmm, mm-hmm, okay. This is another assay testing related question. So, prior assays detected lower levels of CSF HS reduction, the prior assays that detected lower levels were not ideal. Based on new assays, what threshold of reduction do you believe is necessary to support evidence of effectiveness for these contemporary therapies in development?

Dr. Joseph Muenzer: So from my perspective, you probably need at least maybe 50%, but that number is really soft. Only time will tell what we really need and that's why we need to use decreased biomarkers. Again, not every trial in the future is going to succeed, and so we have to be ready to take the regulatory flexibility, get treatment early to these patients and time will see what really makes a difference. But I think 5% or 10% is not enough, but as we already heard, that may be enough to help patients. But for long-term sustain, we may need higher levels. But I think that to be determined yet.

Susan Winckler: Maria, yes?

Dr. Maria Fuller: Thank you. If I can just add to that, I think one of the things that's critical too is that the patients are all being treated at different stages of disease. We know these are progressive disorders and we know that the heparan sulfate accumulates with age. So, you're going to have to have an individualized response about a patient that started with what their baseline was to what impact the treatment's having. And I think from the data that's out there, all of them are showing a dramatic ... I mean, the ones that are, it's between 50% and 85% reduction. So I don't think we're seeing ones that only have 5% or 10%. They are significant reductions. But if you look at the baseline data, that is all different for the different patients as well. So I think that's where reference intervals are going to become important. And one of the steps in the validation is reference intervals.

Susan Winckler: Yeah, Mark, please.

Mark Dant: I just want to add one more time that if you remember Sadie and Cole, stabilization is key. Stabilization is a win. It's nice to focus on a cure. We don't use that word. Stabilization is a win. And I would hope that all of our partners at the review teams, and the FDA, from the top to the bottom and back up again, will remember we in the community of MPS understand buying more time for better science is possible when we stabilize the condition.

Susan Winckler: So, let me pick up on that a bit, because I think it gets to your observation about the challenges in a double arm study. And so, if there were a single arm, you'd still have to measure a response to show that it's clinically meaningful and that holding your own. Just thoughts on that.

Mark Dant: Sure.

Susan Winckler: It's part of this measurement challenge, right?

Mark Dant: The current quandary is we seem to be set in the ... we must measure clinical response through testing. We have a problem with testing as Cole demonstrates. How do we climb that hill? Well, we also know that sometimes these rare conditions, like MPS, take a long time to show clinical difference. Do we run a trial for five years or 10 years? The answer is no. Because when we do that, we see that our biotech partners, many of them, startups, don't have the cash burn to stay afloat for five or 10 years. So what happens to the transformational science? It goes back on the shelf. We have a great one for MPS I. Data was presented at a world two weeks ago. It was remarkable, a video of this little boy. Everything changed with gene therapy for this little boy. Do we wait five or 10 years? Probably not. I think when we rely on the science itself, the transformational science of heparan sulfate, the scientists will tell us what to do.

Susan Winckler: So, one of the regulatory components, and I was triggered by your five to 10-year mention, right? One of the statutory requirements in accelerated approval is then the post-approval confirmatory trial. That's a component. So as evidenced, do you have thoughts about the five to 10-year post-approval confirmatory trial when we may have ... How should we deal with that in some of the things that you exposed about participation in the assessments?

Mark Dant: Again, when you open the trial to more than six participants, or 10, or even 42 in the second trial for MPS-I, you have an entire market who then can ... Your broad base becomes even more broad in that all the patients then have the opportunity to present with time and the companies, then collect the data, and bring it back to the FDA as is the requirement. I think this system works. We just have to let the system work.

Dr. Joseph Muenzer: Let me respond to that. It's important to realize that these patients with five or 10 years of observation will very clearly see the difference. We don't need sophisticated tests. It'll be quite obvious just to any observer who has benefited, because historically these patients really rapidly progress, lose skills. And if we can just stabilize them, it'd be very obvious from the natural history curve in three to five, even 10 years, we'll see a dramatic change. But again, we're treating patients in this time, we're not waiting. Yes, if we waited 10 years, do a trial, we would see what was happening and we'll see benefit. But we're sacrificing a whole generation of individuals to prove the point that heparan sulfate is a reliable biomarker for CNS disease.

Susan Winckler: So then, this is back to you. It's labeled as such. So, you can still deflect it, but it's labeled as such for you. So, how do you explain the discrepancy between heparan sulfate levels and phenotype, increased heparan sulfate levels in slow progressors and lower levels in rapid progressors in the natural history data presented?

Dr. Joseph Muenzer: I'm not sure. The question is how to explain the difference? They have differences. Their biological variabilities. As I said, probably the amount of residual enzyme is the driver. If you have more residual enzyme, you have less of the distal material. They all have stored material. It doesn't matter whether it's slow or fast, it's just that they progress at different rates because of the heterogeneity disorder. And that's what is the problem for clinical trials when you only do it for a year or two, with a placebo-controlled arm.

Susan Winckler: Yeah, then separate, but related. Do you have or are you aware of data on the levels of heparan sulfate in neuronopathic versus non-neuronopathic MPS?

Dr. Joseph Muenzer: There's been some data presented showing that even the non-neuronopathic patients have elevations of heparan sulfate, but it's just nowhere near as high as the classic neuronopathic patients. For example, in MPS-II, there's data supporting that. And I showed a slide with MPS-IIIA. That's showing that there is some suggestion that the slower progressive patients have less, but they still have elevated, it's still causing disease, and just takes longer to progress.

Susan Winckler: So then, this is back to, I think, the case example you raised Dr. Fuller, the example of a pathogenic variant not causing disease. Could you envision newborn genomic sequencing identifying patients at possible risk and then close monitoring with earlier confirmatory biochemical testing to identify at-risk patients earlier? So next time, we're going to let Dr. Fuller go first, but all right, go ahead.

Dr. Joseph Muenzer: No, I'm happy to take this question, because again-

Susan Winckler: We know that, but go ahead.

Dr. Joseph Muenzer: The point is that likely pathogenic with a laboratory error, they didn't know enough about that variant to call that. The ability to predict variant severity is really still somewhat obsolete. And even though they think they can do a good job, it's really that GAG storage, as you heard it, the key event. If you don't store GAGs, you don't have MPS. MPS, basically the diagnosis of deficient enzyme and elevation of the stored material heparan or dermatan sulfate. And you don't need the molecular test to do that. I would argue not to do the molecular test, because there's so many variants like we just recognize that would get flagged and you would caught unnecessary burden. The idea that we're doing now primary enzyme testing and then using the Fuller method with endogenous gags to rule out those pseudo deficiencies and only identify patients with clinical

disease based on elevated non-reducing ends and dodges non-reducing ends works beautifully, and should be the standard of care in the country.

Dr. Maria Fuller: It'd be great if I disagree with you, but I don't. I think the newborn screening by genomics is coming. I think it really is. There's a big push for it. But I think the clinical geneticists don't really quite understand the importance of the biochemistry. And I think something like Hunter is really difficult because most of the variants are private. There are hundreds. The ACMG guidelines, although they've been revised of late, we just do not have enough tools to estrogen pathogenicity at the moment. So I think it's dangerous doing it by genomic technologies until we have better predictive tools. And one of the most powerful ACMG guidelines is the biochemistry. If you can use PS3 or PP4, you pretty much can say it's pathogenic. And that then comes problematic when you're looking at enzyme activities. Because again, we don't really know how much is enough to avert disease. And as Dr. Muenzer said, I think the enzyme is the first tier, the oligosaccharides is the second tier. And yes, we'll do genetic testing afterwards because you obviously need it for cascade testing within families. But I think to switch the paradigm the other way, quite frankly is dangerous in the current climate.

Susan Winckler: Mm-hmm, okay.

Dr. Joseph Muenzer: I absolutely agree.

Susan Winckler: Okay, so we want to know earlier, but not by that, not that structure. All right, a question from online. Is there an initiative to certify a lab for GAG quantification that all startup companies with limited resources might use? And then something I'll just put out there, because we can't answer for FDA. Would FDA be interested in supporting or validating a commonplace facility for companies in this space? So first question, is there? And maybe that's a, should there be? Oh, now she's tossing it to you, so you do have to take it.

Dr. Joseph Muenzer: I don't think there should be one lab. There should be multiple labs doing this technology, and so they have redundancy, and they can validate it, but the method works.

Susan Winckler: Mm-hmm, mm-hmm, okay so then it addresses the questions that we heard earlier from Dr. Marks about the components that are required there.

Dr. Maria Fuller: Sorry, I'll just add, not that I'm going to disagree with you. I think cross-validation is important, and I think these external quality control programs that ERNDOM and things provide where they send out samples to all laboratories. Oh, the European reference for neuro metabolic diseases, I can't remember. I did have the acronym up there, but it's the reference for metabolic disorders and they send out reference material blinded to all laboratories that want to achieve accreditation. And I think that's a good ... So, if laboratories are proposing to do work for validated biomarkers, then one of the criteria is

participation in these external reference programs, and you get the marks back whether you've got the diagnosis or the concentration right, and it's a way of measuring proficiency and ongoing performance. And I think to Dr. Munzer's point, we got into a bit of a strife in Australia where we couldn't get the enzyme. So, I think it's important to have multiple labs that are all validated, so there is some redundancy in the system and also the opportunity for cross validation. So in the past, we've sent samples to Great Ormond Street. They've sent samples to us where we've had difficult cases. And I think those sorts of things are really powerful. If you have one laboratory, you're going to not have the redundancy and make that sort of cross validation possible. So, I think that's important.

Susan Winckler: And then the last one that I have here, but I'll see if there's any more paper that comes up to the front. So, it seems from the questioner's observation that we have safe gene therapy programs stalling to address at PS3. Thoughts on how we might break through that, recognizing that you've all offered some thoughts on this and none of us on stage are decision makers. And so, this is just for input and thoughts.

Dr. Joseph Muenzer: Well, we just explained in the PS3 lectures why we could use biomarkers to push the process with long-term followup to show clinical efficacy. They're safe, we have the initial safety data, and now we need to show clinical efficacy. Doing clinical trials, really not the way to go because these are too slow in terms progressing, but using biomarkers and then requiring companies to do five or 10 years of long-term, treating patients, and using that much larger population to really demonstrate that gene therapy can work. As we probably heard, not every one of these trials may work, but that's the nature of accelerated approval. There are probably some risks should be involved, and the FDA shouldn't consider they have to have 100% response to approve accelerated approval.

Mark Dant: We have the authority, we just have to use the flexibility. Those four words, families go back to constantly, our scientists do as well. We would want our FDA partners, our review teams to go back to them as well, reasonably likely to predict. It's not beyond a reasonable doubt. It's a completely different standard. Reasonably likely to predict, that's it.

Susan Winckler: And then, there are ... It's so really important. And that is where we also see a lot of tension, I think in this case example, saying, "Yes, and we have a reasonable likelihood of prediction potentially," right? And then a lot of tension of ... Certainly, there are a number of skeptics of the accelerated approval program generally. And so that just is a reality.

Mark Dant: It is a reality. And I would ask the skeptic to ask the parent of any of the 85% from 2010 to 2020 ... 85% of all accelerated approvals were for rare cancers. Is anyone complaining about that? No, because we understand that rare cancers need to be treated.

Susan Winckler: Actually a few are. Unfortunately, but yes.

Mark Dant: Well, I think we would have some that might complain about the weather today. But I kind of like it.

Susan Winckler: Yeah, indeed, indeed.

Mark Dant: We have the flexibility, we have the authority. Reasonably likely to predict works very well for many reasons. Maybe focus on the patient.

Susan Winckler: Yeah. With that, Mark, I'm going to let you have the last word there in saying the focus on the patient, because that helps us as we think through in all the work that we are doing in this area. So, that takes us through our first section of the program. You have 32 minutes to break. For those of you who are in the room, there will be lunch outside. For those of you who are joining us virtually, we will be back in 32 minutes. Thank you all.

Mark Dant: Thank you.

Lunch Break