National Organization for Rare Disorders

Workshop on Immune Responses to Enzyme Replacement Therapies:

Role of Immune Tolerance Induction

June 9, 2014

FDA White Oak Campus, White Oak Conference Center
Building 3
Silver Spring, MD 22230
The Great Room

P A R T I C I P A N T S

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C O N T E N T S

P R O C E E D I N G S

Opening Remarks

DR. GRIEBEL: Good morning. Hi everyone, welcome. Trying not to be too loud here. Welcome everyone to today's workshop, which will examine the role of immune tolerance induction in the context of enzyme replacement therapies. I'm Donna Griebel, I'm the Division Director for FDA's Division of Gastroenterology and Inborn Errors Products, and I wanted to open today's workshop by first extending our gratitude to our co-sponsor, the National Organization of Rare Disorders, for their contribution to helping make this workshop a reality. In addition, I want to thank our steering committee members for all their hard work pulling this agenda together. They include representative from the patient community, academia, industry, NORD, and government.

We're here today to explore an important topic that's scientifically exciting and it's challenging. Development of enzyme replacement therapies for rare diseases has represented a major scientific hurdle that we're clearing; however, we're aware that patients may develop drug neutralizing immune responses that can result in impaired efficacy and loss of efficacy. These diseases can be devastating, and we can't ignore opportunities to

actively seek effective interventions for immune responses that result in loss of the gains achieved through important developmental work that have made these therapies possible. In addition, if we can successfully develop an effective means for altering the neutralizing immune response, we need to consider how and when that intervention should occur. Should it be prophylactic, and if so, under what circumstances?

The presentations and your discussion today are critical on a number of levels. Number one, this is an opportunity to potentially heighten an awareness and stimulate research. Number two, it's an opportunity for sharing knowledge, and to facilitate ongoing research. Three, it's an opportunity to identify knowledge gaps, and the means and strategies fulfilling those gaps. And finally, you're helping FDA and industry understand the stakeholders' position on the need to encourage this research, and whether the knowledge basis is currently adequate to potentially make this a systematic part of ERT development programs. If the latter is the case, should it apply in all circumstances, and what are the criteria for making it so? And has the research paradigm been adequately defined to implement an immune tolerance research algorithm so to speak, as part of ERT development programs. If so,

what is that paradigm?

These are a lot of questions, however, I would like to make it very clear that this is not an advisory committee meeting. We're here to be part of a scientific discussion to explore what is known, what isn't known, and to identify key unanswered questions and to identify information resources for finding answers to those questions. With that, I would like to turn this morning's meeting over to Doctor Pariser, Doctor Kishnani, our moderators for this morning's session.

Session 1: Immune responses to enzyme replacement therapy (ERT)

DR. PARISER: Thank you very much, Donna. I'm Anne Pariser, I'm the Associate Director for Rare Diseases in Cedar, in the office of new drugs. And I'd also like to welcome everybody today, for those of you in the room, and also for those of you joining us on the web. We have a really exciting meeting for you today, and as Donna knows, this is really an important and timely topic and we're really glad that everybody could come and participate. And as Donna mentioned also, this is a workshop, it's not an advisory committee, we really welcome everybody's opinion, we'd like to hear from everybody, and we've left a lot of time for questions and discussion and we'd really like to hear from you.

So, just a couple of logistical issues before we get started. The agenda, the slides, the bios and the disclosure should all be posted up on the web on NORD's website. There's a piece of paper with the link out front so if you don't have that, please pick one up at the registration table so you can get to all the conference materials. We have three speakers that's we'll start off with. Doctor Amy Rosenberg, Doctor Barbara Burton, and Doctor Rekha Abichandani, and we ask that, we'll take a

couple of clarifying questions from the speakers, but otherwise, if you could please hold your questions until the panel discussion and then we've left a lot of time for question and answer after the break. So just so we have a chance to hear from all our speakers first. And, so, with that, I'll ask Doctor Kishnani if she'd like to say a few words?

DR. KISHNANI: Good morning, and really wanted to thank the organizing committee, NORD, for putting this session together. I think this is an area of very important unmet needs amongst our patient community and populations, where we've made so many advances in the arena of enzyme replacement therapies, and I think this is a challenge that we face in the field and we are still grappling with how to identify if it truly is a challenge, and then the next is how do we impact and try to prevent this kind of immune cascade. So without much ado, thank you to everyone, good morning, and I think this is going to be a very informative session, and I'm hoping there's a lot of exchange between the speakers and the audience, because that's what makes a good meeting. Thank you.

Impact of anti-drug antibody (ADA) development on ERT: FDA experience

DR. PARISER: Okay, so I'd like to introduce, then, our first speaker. Our first speaker is Doctor Amy Rosenberg. Amy is the Director of the Division of Therapeutic Proteins in the Center for Drug Evaluation Research here at FDA. She has been directing this division since 2000, and is an FDA veteran, having been in FDA for more than 25 years. Amy's background is in immunology and internal medicine, and she's also the FDA advisor to the Immune Tolerance Network, which makes her uniquely qualified to speak on the topic, so welcome Amy, and thank you for speaking.

DR. ROSENBERG: So thank you, and thank you to all of the groups that are participating today, and a special thanks to Jessica Lee, whose backbreaking work I think was critical in advancing this workshop. So, we're going to discuss the effects of immune responses and of immune tolerance reductions on those responses, in enzyme replacement therapies for lysosomal storage diseases. I have nothing to disclose, working for FDA.

A few definitions I think would be in order, to start with. We talk about immunogenicity a lot, and we really define that as the propensity to generate immune

responses to the, in this case, enzyme replacement therapy, or other therapeutic proteins, or the propensity of, to induce immunologically related adverse clinical events. You'll hear a lot about CRIM status, and CRIM refers to Cross Reactive Immunological Material, and I will get into that definition in more detail later. As far as immune tolerance, you will hear a lot about that today, and I'm going to use Doctor Turka's definition, which is the absence of a deleterious immune response, without the need for ongoing therapeutic intervention, very critical.

So, I thought it would be useful to start by discussing the players involved in generating these immune responses. And so, what you can see is that the real lynch pin in generating antibody responses to enzyme replacement therapy is the helper T cell, and the helper T cells can be activated by the therapeutic enzyme being taken in by professional antigen presenting cells, processing and presenting them in association with MHC Class II, that can activate the helper T cell. The interaction between the helper T cell and the B cell is absolutely critical for driving B cells, which are specific again, they have specific receptors of the enzyme replacement therapy, the helper T cell drives the B cell to proliferate and to differentiate, differentiating into memory B cells,

differentiating into antibody short-lived plasma cells, or long-lived plasma cells. And we don't' really have time to go into all of the critical interactions, hopefully some of these will come to light during the talk.

So, how does antibody block the efficacy or the activity of enzyme replacement therapy? Well, there actually are several mechanisms by which this happens. So, first, just to show you, enzyme has an uptake domain, which for most of the diseases we're talking about today, is specific for the mannose 6 phosphate receptor, and these consist of mannose 6 phosphate-targeted sequences, and so the enzyme gets taken up by these receptors, it gets into the lysosome, where it processes substrate, okay? If there's an antibody specific to the uptake domain, we call this neutralizing uptake antibodies, it blocks the ability of the enzyme to get in through the mannose 6 phosphate receptors and it never gets in to process the enzyme. There are antibodies to the catalytic or activity domains, and the enzyme, in this case, can get in through the mannose 6 phosphate receptor, but either its processing is blocked in the lysosome, or because it's got antibody to the catalytic domain, it fails to process the substrate.

Now, in the case where you don't have specific antibodies to these domains, but you have high titered and

sustained antibodies, that can block the efficacy or the activity of the enzyme replacement therapy by diverting it away from target cells that expressed the mannose 6 phosphate receptor to cells like monocytes and macrophages that express FC receptor, okay? So the FC receptor binds to the FC portions of these antibodies, and it degrades the complex, and actually, it facilitates immune responses, so those are the three ways in which antibody can block the activity of enzyme replacement therapy. So, for all of our therapies, we do a risk analysis for immunogenicity, so we're now going to do such for the risk of immune responses across the spectrum of lysosomal storage diseases.

Now, we know a lot about the likelihood of generating immune responses, because we can measure that, and we do a very good job of that in general. So, we can measure the antibody response, what we aren't sure about, in some cases, are the consequences of that response, but in some situations we do. So in some lysosomal storage diseases, the consequences of the antibodies and the antibodies blocking the enzyme replacement therapy are well known and you will see this with respect to pompe disease, and in terms of other lysosomal storage diseases, the consequences of the antibody blocking the enzyme replacement therapy are relatively poorly defined, and that's for fabry

and MPS1, and we'll go into more detail in that later.

So, the critical thing is how we act on the immunogenicity risk assessments. It's predominantly the severity of the consequences of the immune responses to the therapeutic protein that determines the course of action, and clearly, when the consequences are life threatening, tolerance induction is indicated, or the patient will die. When life threatening immune responses can be predicted, based on genetic mutation or CRIM status, prophylactic tolerance protocols are indicated. They incur much less risk than either very poorly treated lysosomal storage disease, or the more prolonged therapeutic immune suppressive tolerance induction regimens. Tolerance induction may also be indicated when the immune response abolishes the efficacy of highly effective, but not necessarily lifesaving therapeutics. One example would be the TNF blockers, which is not at discussion today. Of course the risks associated with the tolerance regimens, and the impact of the tolerance regimen on the underlying disease itself should be considered.

So let's start with a severe consequence. The antibody response abrogates the efficacy of a life saving therapeutic, okay? And for this, the foundational experience is with Pompe disease, and we will really go into

Priya's groundbreaking work on both the effects of the immune response and tolerance induction. So, as you know, infantile Pompe disease is a lysosomal storage disease, due to the deficiency of lysosomal acid alpha glucosidase. It can be a rapidly fatal disease, primarily affecting cardiac skeletal muscle. Death usually ensues within the first two years of life, from cardiac and respiratory failure.

Now because of the rapid progression of this disease, the efficacy of enzyme replacement therapy, as well as the effects of immune responses to the enzyme replacement therapy are very clear. You see heart failure, weakness, requirement for invasive ventilation, and this is very different from what we see to the more slowly progressive lysosomal storage diseases that have more distant end points. For treatment, recombinant human gaa is the only available therapeutic, and unlike in many of the hemophilias, there's no bypass or salvage therapies here.

So, now let's discuss CRIM, because, as I said, this will come up a lot. CRIM stands for Cross Reactive Immunologic Material and it is an assay, it's an assay that detects the presence of endogenous enzyme by using an antibody specific for that enzyme. So this is the enzyme present in the cells of the patient, so for CRIM positive patients, they have gene mutations which may destroy the

ability of the enzyme to act, but none the less, it still allows for the production of some cellular enzyme. Now, exposure of the immune system to whatever amount of cellular enzyme is there, may tolerize the immune system. So the immune system may see this as self, recognize this enzyme as self. Usually these patients make low antibody titers, but, as you will see, there are some cases of very high antibody titers in this population, and these patients mostly have a very good clinical response, those that make no or low titers, but some cases of, in patients with high antibody titers, the outcome can be poor.

So in contrast, CRIM negative patients have two deletrarious genomuatatations, they are unable to form native enzyme protein at all, they're a knockout phenotype. And in this case, the enzyme replacement therapy that you give back is recognized as foreign, because the cells don't have any recognizable enzyme. These patients usually make high persistent antibody titers, and frequently neutralizing antibody, and they mostly have a poor clinical response to the enzyme replacement therapy. And those points are illustrated very nicely in Priya's slide here, which is that the high antibody titer, and not the CRIM status per se, confers negative clinical outcome.

So if one just looks at overall survival here, the

low titer CRIM positive patients enjoy a relatively sustained response. However, the CRIM negative, who view the enzyme replacement therapy, these are patients on enzyme replacement therapy, who view it as foreign, make antibody responses that nullify the effect of the enzyme replacement therapy and they have tragically early deaths, as do the high titer CRIM positive patients. So, I'm going to just summarize the experience with Pompe, because Priya is going to go into it with much more detail, but this was our foundational experience with enzyme replacement therapy and what the immune response can do to alter that.

So, in summary, I've showed you that the sustained high titer neutralizing antibody responses, abrogate the efficacy of ERT and CRIM negative and some CRIM positive patients. So, the sustained high titer and neutralizing antibody response inversely correlates with overall and ventilator free survival. In slides I did not show you, the antibody response actually, in individual patients is very temporally correlated to clinical decline. And immune tolerance induction, which diminishes or eliminates or prevents antibodies to ERT also prevents the loss of, or restores, the efficacy of the enzyme replacement therapy in these patients. And, so immune tolerance induction in this, for this disease, and for enzyme replacement therapy, is

essential in the CRIM negative and in the subset of CRIM positive patients who mount high titer antibody responses.

So, potential approaches to tolerance induction are numerous, and ideal would be those that are highly antigen specific, and you will hear her talk about that later, which consequently preserve global immunity, and some of them are listed here. However, antigen specific approaches could be problematic, and are problematic in clinical settings that require immediate treatment, or in clinical settings in which significant and organ damage could occur during a very prolonger tolerance induction protocol.

So, antigen specific approaches may take weeks or months for the induction of durable immune tolerance, and organ damage may potentially progress or become irreversible during prolong tolerance protocols, and in situations again, where clinical treatment, and thereby tolerance induction, has to begin urgently, because of deteriorating clinical status. The therapeutic options are really much more limited, and they are targeted, and what you will see, one can potentially target the dendritic cells that initially present antigen, but the therapies you will largely see today focus on therapies that target the helper T cells and B cells involved in these responses with methotrexate that

kills rapidly dividing antigen stimulated helper T and B cells, and may allow for the out growth or the development induction of regulatory T cells. We don't really know the mechanism, and this is an important area of investigation, rituximab is a monoclonal antibody specific for CD20, which gets rid of B cells, gets, abrogates the progression and may really critically influence antigen presentation of the B cell to the helper T cell.

So you'll see a lot more about this and hear much about this in later talks today, and as well as targeting very long lived plasma cells which are particularly difficult. So, the second situation in which tolerance induction is absolutely indicated for a lifesaving therapeutic is when there's anaphylaxis, a severe allergic response to a lifesaving therapeutic. We see this in respect to highly efficacious proteins of nonhuman origin, as well as in CRIM negative patients with ERT. It's not very common, fortunately, but it's much more common in factor 9 in hemophilia B.

So, what have we learned from treating anaphylaxis to factor 9? Well, for factor 9 you can avoid by bypass therapy, we don't have that option with Pompe disease. You can desensitize. Again, it's not an appropriate strategy where you don't have a bypass therapy, and actually, this

comes down to tolerance induction, which is increasingly being using in the setting of factor 9 and their newer approaches seem to be quite effective. Anti CD20, which is the rituximab, seems to work in many situations, and as well, the therapeutic omalizumab, which is an anti IGE monoclonal antibody, should be considered for all IGE positive patients who develop anaphylactic responses to enzyme replacement therapies. Gene therapeutic approaches also have been shown to be effective.

So, I would just like to illustrate this point with this case study, where a patient with IPD on enzyme replacement therapy, experienced persistent anaphylactic responses. They weren't' controlled with corticosteroids, antihistamines, or decreased infusion rate, and this patient was IGE positive. And so, this patient was treated by adding omalizumab and this allowed the patient to continue to receive enzyme replacement therapy. And all the other therapies for anaphylaxis were weaned, and strikingly this patient never developed a high titer IGG response, which I think is matter intriguing, suggesting that the anti IGE treatment may have prevented the class switching, which goes -- which proceeds in B cells. So, very often, when one reads papers where patients have had to cease getting a lifesaving therapeutics because of anaphylaxis, and this

really, I get very disturbed by that because there are mechanisms and treatment protocols that one can use to try and prevent these sorts of responses, so the patient can receive a lifesaving therapeutic.

So, I've just illustrated to you where antibody responses are really destructive. There is something rather interesting though, on the reverse side, and it's a conundrum. So, neutralizing antibodies, specifically neutralizing antibody, may not always diminish product activity and efficacy, sometimes it can act as a chaperone or carrier for therapeutic proteins. It can enhance the P.K. and potentially product activity and efficacy, and we certainly see this with respect -- Fred Finkelman described this with respect to cytokine therapy and their ongoing efforts in that direction.

So, these kinds of favorable antibody responses, though, they're not predictable, they're not controlled, and it may represent a metastable or an unstable state, so that, with some trigger, it would become a response that would, in fact abbregate efficacy. But what this does do, is suggests a novel approach to formulation, to try and prolong the activity and the efficacy of enzyme replacement therapy.

So this came to light quite nicely in a study of recombinant human gaa, and these were CRIM positive Pompe

patients, doing, performing a six-minute walk test, and you can see most patients, you know, had a slight increase, or were level, occasionally some did poorly, but there were four stand out patients who did so much better than everyone else, and so the parameters, the immune parameters of these patients were examined, and it turned out that three out of four of these patients had sustained fairly high titers of uptick neutralizing antibodies, and in fact, as you saw, they had an average improvement in the six minute walk test that was far beyond their cohorts. Again, here's a situation where inhibitory antibody, the neutralizing antibody, can be acting as a carrier protein.

This issue came up similarly with the recent approval of elosulfase alfa for MPS 4 morquio syndrome. Again, raising the questions, are neutralizing antibodies friends or foe? In this situation, all of the patients in this clinical trial developed antibody by week four, indicating a really very low state of tolerance to the enzyme replacement therapy. By week 16, nearly all developed neutralizing antibodies that blocked the uptake domain. Interestingly though, instead of enhanced ERT clearance, the clearance of the enzyme replacement therapy was delayed. You can see the area under the curve and the sig [spelled phonetically] max increased about threefold,

and the half-life increased from 7.5 minutes at week zero, to 35.9 minutes at week 22. So this again suggests that in many of these patients, this neutralizing antibody response is acting as a chaperone. Again, it may be a very unstable state, and something may trigger a rather negative effect of these antibodies, and it's not clear what effect this has on the efficacy of this enzyme, and that's because all patients developed anti-drug antibodies, thus the available data really are inadequate to assess the relationship between antibody development and specifically those patients developing neutralizing antibodies to the uptake domain, and therapeutic response. There is also in this trial, a significant incidence of anaphylaxis, about 8 percent of patients, and these patients might needs to be tolerized if routine management is not successful. Again, no patient should be denied ERT who develops anaphylaxis without undergoing tolerance therapy. In patients not experiencing hypersensitivity responses, again, these favorable antibody responses unpredictable, uncontrolled, and may not be stable, and the question arises, should tolerance be considered in this patient population?

Okay, so now we're going to get to lysosomal storage diseases, where the effect of the immune response on clinical outcome is less clear, so I think I've illustrated,

and Priya will certainly go into great detail, that the clinical manifestations, the effect of ERT and the effect of the immune response to ERT are very clear in Pompe disease with loss of motor milestones and cardiac failure, that the effects of ERT on clinical manifestations and the effect of immune response to ERT in the more slowly progressive lysosomal storage diseases are not clear in many cases, and the clinical endpoints may be reached only after many years. And unfortunately, validated biomarkers to predict clinical efficacy are really lacking for most of these lysosomal storage diseases. Again, we're in a more ambiguous area, because we don't' have a good handle on the consequences of the immune response here.

So, just as an illustrative example, I'm going to talk about MPS 1, because there's not enough time to go through many others. This is a deficiency of alpha eligioranodase leading to accumulation of heparin dermatan sulphates. In lysosomes, the spectrum ranges from very severe hurlers to attenuated disease in she, and an in between syndrome. Hurlers presents very early, birth to two years, rapidly progresses with terribly clinical outcomes, death within a decade if untreated. She -- the onset is delayed with little or no neuronal involvement, and again, the treatment is enzyme replacement therapy with alpha

eligioranodase and as well, allegiance bone marrow transplantation seems to be the new standard of treatment for these patients.

But when one examines the antibody outcome in these patients given enzyme replacement therapy, in the very young ones, the CRIM negative patients, they make stronger immune responses, you see some decrease in substrate reduction, some decrease in urinary gag reduction, which is a measure of the substrate and the ability of the enzyme to get rid of that substrate, and you see a decline in titers, but many of these patients, as you can see here, I maintain, first of all, the [unintelligible] can work very rapidly, and they maintain high sustained titers, which we know has a -- can have a profound effect, probably mostly by diverting enzyme into those cells which don't really -- are not really the target cells here, the critical target cells, into macrophages and monocytes. Now, the -- in this case, the antibodies to the enzyme replacement therapy are associated with increased substrate levels. So, if you -- this is antibody titer on a log scale, the y-axis here. And what you can see here is in patients with high antibody responses, their percent reduction in urinary substrate, so, in essence, the ability of the enzyme to digest substrate and target tissues is quite low, in some cases it's very

low. It's variable, but it's low in general. This compared to patients with lower antibody responses who have a much more substantial percent reduction in substrate indicating better activity of the enzyme in target tissues.

So there is an inverse relation in this case of disease biomarkers with antibody response, and awhile back it was asked whether elimination of the antibody response improved the efficacy in CRIM-negative NPS. And, fortunately, there's an excellent animal model for NPS one. It's a spontaneously arising dog model, and these dogs are CRIM-negative, and so what you can see here is that when you give these NPS one dogs the enzyme replacement therapy, most of them develop high titer and neutralizing antibodies. But, they can be tolerized, and so this protocol was a Cyclosporin A based protocol, and these dogs were rendered tolerant to the enzyme replacement therapy. Well did the tolerance increase the activity of the enzyme? And the answer is yes.

This is a lovely study from Patricia Dickson who's here today, and what you can see here is the amount of enzyme activity in the critical target tissues in the tolerant versus non-tolerant dogs. And what you can see is that the percent change of tolerant versus non-tolerant in critical target tissues, kidney, long, heat-valve,

myocardium cartilage, is much better in the tolerant dogs than in the non-tolerant dogs. And of particular note, you can see very high uptake in the non-tolerant dogs in the liver and spleen of this enzyme, and that's because these are tissues that have very high levels of those macrophages and monocytes that have FC receptors that -- to which antibody binds, okay? And you see a relative -- much less of that in the tolerant dogs. So in this situation, the enzyme is getting to the critical target tissues in the tolerant dogs, but not in the non-tolerant dogs.

Animal studies have shed light as well. This is an antibodies to alpha-galactosidase to treat Fabry disease, and what you can see is that the in mice, these are knockout mice whereby you add patient sera that are seropositive to the enzyme versus having just plain serum, you can see that there's a dramatic reduction of enzyme activity in the kidney as well in the lung in the presence of antibodies as opposed to the activity and the lack of those antibodies.

So, my conclusions and questions here is that there is a preponderance of evidence, and it's a substantial preponderance of evidence that indicates the persistent moderate to high titer or neutralizing antibody responses, except in rare circumstances, interfere with enzyme replacement therapy, uptake and or activity in critical

target tissues, and thus very likely diminish efficacy in those diseases for which we don't have a great handle on clinical outcome. Prophylactic immune tolerance regimens, as you will see, allow for unimpeded enzyme replacement therapy activity, eliminate the potential for a substantial antibody response to allow for irreversible tissue damage, and reduces the intensity and duration of immune suppression associated with therapeutic tolerance induction protocols to reverse a high titer immune response. And, as you will see, there's a very favorable safety experience with these prophylactic tolerance induction protocols in Pompe which, in some ways, is really a worst case scenario.

So given that, should tolerance induction be considered in all CRIM-negative patients with lysosomal storage diseases? In addition, you know, we have to always think about the future. Immune tolerance to enzyme replacement therapy may have some very clear additional benefits, reducing or eliminating the immune response to potentially more efficacious therapies, biomarin enzyme replacement therapies, genetic therapies, and exon skipping approaches. Moreover, establishing tolerance to the enzyme may well diminish immunogenicity and enhance engraftment in the setting of the allogeneic bone marrow transplant that is used increasingly for Hurler's.

So in this situation, you have already primed T cells, T cells primed to see the peptides of the enzyme in association with HLA. So the question is, is would removing that immune response enhance engraftment, because we do see graft failures in some of those settings, and I would just like to acknowledge, just in particular, my FDA colleagues. I am, again, very grateful for all the attendees today, and really hope that we get great discussions of the issues at hand today, so thank you very much.

[applause]

DR. KISHNANI: We can take a couple of questions right now, and there's also going to be an opportunity at the end of the panel discussion. If there are any questions, if you could come up to the mic, please.

MALE SPEAKER: So Amy, you were talking of circumstances in which having an antibody titer was actually efficacious in terms of being able to maybe reduce the -- but you talked about this neutralizing antibodies. Have the epitopes been mapped? Are they truly neutralizing? Or do we know where on the recombinant protein these antibodies bond? Is it different in different patients?

DR. ROSENBERG: Yeah, it's a great question, especially in view of the use of that kind of neutralizing antibody. The IL-2 to serve as a carrier for IL-2, that is

not currently known to my knowledge. What is known is that it blocks uptake in an uptake assay. So you're right, it would be of great interest to see, particularly, where neutralizing antibody actually facilitates enzyme -- the activity of the enzyme. That's a great -- that's a great research question.

DR. WHITLEY: Hi, Chet Whitley, University of Minnesota. The elosulfase information was very interesting. I would presume that's not published; at least it's new to me. The fact that all patients developed antibody is quite remarkable, although is there any evidence that it's really impacting clinical efficacy. It seems like it might be a big jump, in fact, counter-intuitive, to think that increasing the area of the curve is going to improve efficacy if there's more enzyme sequestered in the plasma, it's not going into those critical target cells of the bone and cartilage.

DR. ROSENBERG: Right, but what may be happening is the -- and I have a feeling it has to do in part with the affinity of the antibody -- that's another thing I think we need to explore is the affinity of those antibodies. They act as a carrier and that enzyme will dissociate. So instead of being rapidly taken up, perhaps by many non-target tissues, you get a slower dissociation. So it --

there is, from that study, there's no evidence that the antibodies have an affect. At least on the biomarkers measured.

DR. WHITLEY: So there was no evidence positive or negative that antibody was associated with efficacy?

DR. ROSENBERG: Yeah, I think it's impossible to know especially since everybody makes those responses. Thank you.

DR. KISHNANI: One more question, and I think we'll need to move on, and then we can during the panel discussion. I think, yes.

DR. ROSENBERG: Sorry. I can't hear you. Why don't you come up to an active one?

MALE SPEAKER: Does this one work? Yes. Thanks. Can you comment on the dosing and timing of the administration, whether that affects the tolerance or not?

DR. ROSENBERG: I'm sorry? Okay. Hang on to that question. We're going to get into that. Thank you.

ADA in patients with lysosomal storage diseases (LSD): Clinician's perspective

DR. KISHNANI: Thank you so much, Dr. Rosenberg, and we'll just move on to our next speaker, Dr. Barbara Burton, a very close friend and colleague. She's a Professor of Pediatrics at Northwestern University Feinberg School of Medicine. She's certified in pediatrics, clinical, and biochemical genetics, is a guru in many newborn areas of metabolism, and I know has a real passion for the lysosomal storage disease, so welcome, Barbara.

DR. BURTON: Thank you, Priya, and it's my pleasure to be able to participate in this meeting today. I really do think this is such an important topic as the other speakers have mentioned, and certainly it's something that I have a strong interest in. So I, too, would like to thank the organizers. Here are my disclosures. I have worked with another of the pharmaceutical companies involved in the development of drugs for lysosomal storage disorders, and we're stuck on that slide, I think. I'm trying to get it to move -- oh there we go.

Okay, so I was asked just to give a talk briefly from the clinicians perspective about what the concerns are of clinician taking care of patients with lysosomal storage disorders, and using enzyme replacement therapy. Why would

we care about whether our patients are developing antibodies? What are the issues that might cause us concern? And, I think, there really are two big ones. One is the one that's already been discussed to some extent, and that is that we worry that if patients are developing antibodies, that these would result in loss of efficacy or a negative impact on the outcome of therapy. And, secondly, we worry about adverse drug reactions. The folks at the FDA have encouraged me not to use that vague term, infusion related reactions, and I know from reading the draft document that they circulated, that they'd like us to get away from using that terminology, and use more specific terms like anaphylaxis or cytokine mediated reactions to describe what happens, but we certainly do have many patients who experience adverse events during the course of receiving infusions for enzyme replacement therapy. And, of course, we think that many of those are probably mediated by antibodies. Not all, but they tend to peak during the time when we think that the antibody response is peaking, in most cases. And so, we worry a little bit about the relationship of these adverse events to antibody development.

I'll start by making a few comments about this, because I think, in clinical practice, really, this is the lesser of our concerns from a practical standpoint. So what

is the relationship between antibody formation and these adverse events occurring during the course of infusion, so right around that time. The relationship is really not very clear. Here, you're looking at some compiled data from publications on some of the disorders for which we have enzyme replacement therapy, and I'll direct your attention mainly to the columns that show the proportion of patients with infusion-associated reactions.

On the next to the last column -- I don't know if I can use this point, but the next to the last column, which varies anywhere from about 13.8 percent for Gaucher disease. With Serrazume, 52 percent with VPRIV down to, you can see 50 percent, 15, 30 percent, and then look over at the far right, the proportion of patients with IgG antibodies, 15 percent for Serrazyme. 1.9 VPRIV. Fabry 68 percent. With the MPS disorders, 97 percent. MPS-1 47. MPS-2 95 percent. Pompe 100 percent. Late on set Pompe. You heard 100 percent for MPS-4A. So really not a clear relationship between the incidents of infusion associated reactions, and the incidents of antibodies. We know from drilling down on this data a little bit with some of the -- it's really hard to get these slides to advance. Push this. Okay.

We know from drilling down on this and a number of these conditions that patients with antibodies are more

likely to have adverse events during infusions, and patients with infusion associated adverse events, reactions, are more likely to have antibodies, but there's certainly not a 1:1 relationship, and in clinical practice, most of the time, antibody testing has not been very helpful in predicting or managing infusion related reactions. Although, there are rare exceptions. We heard about one just in the last talk of the patient with IGE mediated anaphylaxis.

I think in most cases, we do not have real time access to antibody testing so in clinical practice we manage adverse events occurring during infusions in real time. So we've learned, practically, how to use prophylactic medications, how to treat reactions acutely. I have, honestly, in all the years I've been using ERT, never had a patient in whom I've suggested stopping ERT because of infusion reactions. Never, and I treat hundreds of patients. I have had patients where I've done 20- to 24-hour long infusions to get them to tolerate it, and gradually inched up on the time course until we were back to a three-hour infusion, and used lots of pre-meds in the meantime.

So there -- I've always found a way, and I think you almost always can without really much knowledge of what the patient's antibody status is. But, I will acknowledge

that there may be rare cases where knowledge of the antibody status, in conjunction with the help of an immunologist might make it easier or might make it possible. But I've certainly never encountered a patient where, you know, it was necessary to consider stopping, or even do anything other than the usual strategies that we use of reducing -- of lengthening infusion time using pre-medications, or using medication acutely. And that includes patients who've had anaphylaxis, because we see anaphylaxis really in association with all of these at different rates.

Okay, so let me move on to: How does the presence of antibodies impact the efficacy of ERT for the LSDs? I think this is a more significant issue. So I think geneticists, who are the main people using enzyme replacement therapy, and treating patients with ERT, we knew, kind of, that anti-drug antibodies would be a problem with protein therapeutics, because most of us were kind of vaguely aware of the problem with factor therapy for hemophilia. We went through pediatric training, and all of that, and we knew about inhibitors, and so forth, and we knew those were antibodies, so we knew factor eight inhibits were a big issue, and so forth. So we kind of knew that, but we really didn't think this was a big issue, honestly, in the early days of ERT for the LSDs.

I think it was because we got complacent with the success of enzyme replacement therapy for Gaucher disease. Our first drug was serodase, and then serrazyme for Gaucher's, and those are all CRIM-positive patients, basically. I mean patients with type one Gaucher disease, they have residual enzyme activity. You saw on the one slide that there's not much antibody production, we see 15 percent with serrazyme. We see 1 percent with VPRIV. They don't make much antibody, and they do well on enzyme replacement therapy, so really it isn't much of an issue with Gaucher disease, so we just kind of trotted along, thinking well, this isn't much of a problem, they get antibodies, but so what?

Then along came infantile Pompe disease, and that's really what brought the issue to light, I think. And so we started, you know, thinking a little bit more about it, and, you know, maybe scrutinizing our other patients a little bit more as the issue got more attention. But our other patients are a lot harder to evaluate. The infantile Pompe patients, as it's been pointed out, they have a short natural history. The natural history of that disease is very short, so if their therapy stops working, it becomes obvious very fast. You know, they go downhill fast, but with a lot of the other diseases we treat with ERT, nothing

happens very fast. I mean, in fact, the goal of our therapy, pretty much for a lot of them is to see nothing happening. We want to, more or less, stabilize the course of their disease, and we feel like if they're not changing, that's probably a good outcome. If they're not going downhill, we're happy. You know, that's good. You know they're stable. So we don't see much change over time, and we don't really have, you know, good biomarkers as has been pointed out.

But we have a lot of others challenges in looking at the impact of antibodies as well. One is just how do we get the antibody titers, and what do they mean. They're available, generally, only through the pharmaceutical companies that make the drugs. They're not commercially available, and I don't think that people treating patients have good guidelines for when they need to get them, and when they can get them. Sometimes, actually, they're only available to patients who enrolled in registries to follow the clinical course of the disease. Registries being part of the post-marketing obligations of the pharmaceutical companies who make it. ERT patients are all eligible to enroll, but not all patients are enrolled. And, so, a doctor can't just commercially order antibodies for a particular drug, and, you know, we don't really have a good

way of judging the reliability of the antibody testing that we're getting through the drug companies.

Now in talking with the folks here at the FDA as we were preparing these talks, they told me, well, okay, the assays are overseen by the FDA so they should be reliable. So they're reliable. So that -- that was good to know, actually. I was not even aware that they were -- that v was the case, so that was a good thing for me to know that the assays should generally be reliable, but it's hard for a clinician to know how to really judge what you're getting back in terms of the significance. Neutralizing antibodies aren't always measured, or they're not always reported back to us, and sometimes we get some screwy results back. I mean, there's one drug, for example, where I get titers on a patient routinely above a million, high titer antibodies, and the patient's doing fine, and the word that I get back is that that's common, and we're not really seeing a good correlation with efficacy, or we didn't in the clinical trials. We haven't in the registry, and I don't know what to do with that or really what that means.

As I mentioned, the clinical response to therapy in patients whose disease course is very long is very difficult to assess. You know, it's different than patients with infantile Pompe disease, so if I'm looking at a patient

with one of the MPS disorders, for example, you know it can be very hard for me to know if the patient's having a good response to therapy or not. I mean, the reality is, particularly after a certain period of time, they don't change much very fast. I mean, the same is true for something like Fabry disease. It's very hard to determine in the short term to judge, particularly, how they're doing. Maybe five years -- over five years maybe we'll see something, but in the short term very hard to say.

And it's not like Gaucher disease where we have this nice panel of biomarkers, you know, chitotriosodase, and so forth, that we can measure in serum, where we see a nice decline or if we start to see a rise, we know something's not going right. We've got good biomarkers. We don't have that. We have urine GAGs for the MPS disorder, but, honestly, we don't even really known what urine GAGs are measuring. Is that total body GAGs? Or is that what's in the renal tubule? You know, that is a biomarker but we don't really know for sure what it's measuring, and it's the only one we have. So is that allowing us to get a valid biochemical assessment of response to therapy? It's not clear.

So let me give you an example of a challenging patient that kind of illustrates the concern in an LSD for

the impact of antibodies on the response to therapy. This is a little boy that we reported at the world meeting earlier this year that has MPS-2, and had high titer antibodies, and we felt evidence of lack of efficacy of his enzyme replacement therapy. He was a fairly typical patient with MPS-2. His disorder was initially suspected at 17 months of age based on coarse facial features, hepatosplenomegaly, and multiple joint restrictions. It was confirmed by enzyme assay showing deficient iduronate-2-sulfatase activity, and a microarray that revealed a complete dilution of the iduronate-2-sulfatase gene. So based on that, he's predicted to have a severe phenotype because of the complete gene deletion, he would clearly be a CRIM-negative patient. He’s not going to make any enzyme protein. He doesn’t have an I2S gene. This is an X-linked gene, if there are any of you in the audience not familiar with MPS-2.

So the patient was started shortly after the diagnosis on enzyme replacement therapy with Elaprase at the recommended label dose of 0.5 milligrams per kilogram per week. After two years of therapy, there had been no significant decline in his urine GAGs. Most patients within about there to six months have a very significant decline in urine GAGs to near normal, if not normal. He also had no

evidence of a clinical response to treatment. Usually we'll see a decrease in liver size pretty quickly. You may or may not see improvement in joint range of motion, he didn't have any. And no other particular change in physical findings. You may not see too many of the changes, but he didn't have any.

So his dose was increased at this point to one milligram per kilogram per week. After another one year, there was still no significant decline in urine GAGs or change in the physical findings. During treatment, and this was, you know, the reason -- part of the reason that his dosed was increased, he had high titers to begin with, after his dose increase -- was increased, these rose higher. High IgG titers to idursulfase. Prior to immune modulation which I'll show you his IgG was 204,000 with 100 percent neutralizing antibodies. He was positive for IgE with a titer of 160. He had had some infusion related reactions but they were relatively mild and easily treated.

At five years of page, the immune modulation protocol was initiated. It consisted of protocol very similar to what Priya has described with Pompe disease, except that ofatumumab was substituted for rituximab. And at the end of the initial treatment period, he had had only a slight decline in his antibody titer, in his urine GAGs,

so there was some intensification that was done with his bortezomib and dexamethasone was added, and six weeks later, a more significant decline was observed, so he was started on a maintenance regimen at that point.

He had some infusion related reactions, or adverse reactions I should say to the ofatumumab and had to be treated for that. He had some side effects of hypokalemia, edema, and irritability, either from the drugs or from pre-medications that he received. During the therapy, he started having infusion related reactions to the IV Elaprase, anaphylaxis. The dose was eventually reduced to the label dose to try to manage this after we had tried a lot of other strategies, including the usual ones. And he was successfully treated, and then the family wanted to, again, increase the dose back, which we did do successfully and he tolerated it.

Here you see his antibody titers over time extending out from the base line to 18 months. His urine GAGs have not really come down all that dramatically. Normal is around 100 or less, and he's come down from about 500 to about 300, but really has not normalized despite the decline in his antibody titers. The family, however feels like he's had a clinical response to therapy that's been very significant, and his teachers apparently have reported

a significant increase in his walking ability, his school -- they say he's much more mobile at school, walking much longer distances. The parents report the same.

We have had sequential ultrasound examinations that have documented a decline in his liver size -- he's really hard to examine clinically. So, we've done ultrasound to try to get a better handle on liver size. His joint range of motion has improved quite a bit, but the patient's also been receiving elmiron, which is a medication that conceivably could improve joint range of motion. He has had a decline in his cognitive status over this time period. He has a severe phenotype of NPS-2 and we wouldn't expect that his intravenous elaprase would impact that at all. And he does continue to have intermittent infusion related reactions, which he tolerates and the family feels are tolerable -- so we continue to push on with this intravenous therapy. And he continues on his maintenance immune tolerance regimen. That's it, any questions?

[applause]

Assessing immunogenicity during clinical development: Industry perspective

DR. PARISER: Thank you very much, Dr. Burton. Our next speaker is Dr. Rekha Abichandani. She's the vice president of clinical development at Shire, where she's been since December of 2012, and she has responsibility for development of Shire's intrathecal enzyme replacement programs for Hunter, San Felipo and metachromatic leukodystrophy. She has over 12 years of experience in the pharmaceutical industry in developing rare diseases as well as hematologic malignancies. And her background is in internal medicine and nephrology. Dr. Abichandani will be speaking on assessing immunogenicity during clinical development from the industry perspective.

DR. ABICHANDANI: Thank you. I am presenting today an industry perspective on the industry representative nominated by BIO. I am an employee of Shire, and although my presentation has been informed by views of other companies within BIO, it does not represent a formal consolidated position of either BIO or any other companies within BIO.

So let me start out by saying -- presenting what our perspectives are and recommendations. From an industry perspective we recognize that assessment of immunogenicity

and the impact of anti-drug antibodies on clinical outcomes is very important. We recommend an individualized risk-based approach for each treatment and each disease in determining the need for immune tolerance. And we also recommend a risk-benefit assessment when considering immune tolerance induction. So, how do we, in industry, assess immunogenicity? This slide shows a tiered approach for assessing anti-drug antibodies. And ths is in line with FDA guidance.

So, again, typically validated and sensitive assays are developed, we start out with having a highly sensitive screening assay, and if the assay is positive then we follow up with a confirmatory assay. If the confirmatory assay is positive, then an anti-drug antibody titer is reported out. If the confirmatory assay is positive, subsequently, the patient is tested for the presence of neutralizing antibodies. And again, if neutralizing antibodies are positive, it's followed by a tight ring of these anti-drug antibodies. IgE testing should also be conducted in the case of suspected hypersensitivity.

So, how does one establish -- there are a number of strategies for immunogenicity testing during and after -- during clinical development and in the commercial stage. It's important to establish a comprehensive testing scheme

early on in clinical development. It's important to develop assays for use in early phase one clinical trials. Now historically, there's been a lot of variability in how companies have approached this, and there are a number of different assay formats, but they're all sensitive, and -- they should all be sensitive and validated. It's important to have validated assays in time of -- at the start of pivotal trials. A number of companies either already have them or are working towards having these assays in time for pivotal trials.

As I mentioned, the format and sensitivities of these assays sometimes do vary, and the greatest challenge from an industry perspective is the time that it takes to develop validated assays. IgE-specific anti-drug antibodies need to be developed early if you're suspecting hypersensitivity. Enzymatic and uptake or receptor-binding neutralizing antibodies are expected at the time of pivotal or -- pivotal trials. There's been a lot of talk about CRIM. I think that one question, from an industry perspective, is whether CRIM assays should be developed systematically for all LSDs. I think that's not a requirement at this stage, but a number of companies are developing CRIM assays for their products. It's traditionally been done by bestern blot, but I think one of

the questions that also comes up is, "Should other formats be considered?"

There are a number of factors to be considered in the immunogenicity assessment -- and this again based on the FDA guidance for assessment of therapeutic protein products. And I've highlighted here in red the products -- factors that may be important, specifically more important for development of ERTs. There are patient-specific products and product-specific factors. There are some development considerations, and at the end of the day these are orphan populations, so we are starting out with very small data sets. And it may be difficult to determine the impact of these anti-drug antibodies unless there's a very dramatic impact of the antibodies on clinical outcomes.

CRIM status is great, it's a simplified assessment of the immunogenicities risk, however, as has been noted, CRIM positive individuals may also develop antibodies and have poor clinical outcomes. It's important to collect genotype data in the early clinical trials. And, I think, early on in the clinical development some of the challenges are, you know, when -- it may take a long time to demonstrate clinical benefit. And how does one correlate the presence of antibodies with biomarkers -- and if you haven't seen clinical benefit yet, is it due to the presence

of anti-drug antibodies, or is it due to a lack of efficacy of the ERT? And antibody development may not correlate with clinical outcomes.

So there are some key consideration from an industry perspective in developing immune tolerance trials. I think the first one is, you know, what's the level of clinical impact that warrants immune tolerance, and identifying patients who may benefit from prophylactic immune tolerance regimen. And it's weighing the risk-benefit of the immune tolerance -- when is the risk of the disease that is untreated or partially effective therapy outweigh benefit -- outweigh the risk of an immune tolerance regimen itself? And what's the risk of the immune tolerance regimen? And when should immune tolerance trials be conducted? In a relative -- should it be conducted prior to the demonstration of clinical impact -- is biomarker data alone sufficient? And how -- or should we wait for demonstration of clinical efficacy before we conduct immune tolerance regimens. And what is the timing relative to approval, should these be conducted prior to approval, or in the post-approval setting? There are also a number of immune trial regiment under investigation, and is the same regimen appropriate for all diseases -- or for different patient populations within a certain disease?

And I mentioned, again -- these are small sample sizes, so when we conduct these immune tolerance trials we're probably talking about even a further, smaller subset. We are using -- we are considering regimens that -- and dose in duration of agents that are not approved for this indication -- and pediatric patients. So, the complexity of adverse event assessment is more complicated. And what is the impact of labeling when we conduct these immune tolerance regimens? What is the impact of labeling on the agents that are used to immune tolerance -- and I guess one question that we often wonder is, "Would this be considered therapy?" In terms of labeling. We are talking about immune modulating agents, so there may be an added infection risk and some of these enzyme replacement therapies are being explored for intrathecal use -- we are using devices so there may be an added infection risk. And how do we monitor the response for -- to vaccination. And provide guidance to physicians. And, as I mentioned earlier, should these trials be conducted prior to demonstrating a clinical response of the ERT? And there are multiple immune tolerance regimens under investigation. And is there an opportunity to develop a consortium to inform such studies in the future?

So, to summarize, we recognize that assessment of

immunogenicity is very important and we recommend an individualized risk-based approach for each treatment and disease in determining the need for immune tolerance and recommend a risk-benefit assessment when considering immune tolerance induction. Thank you very much, and I'll take any questions.

[applause]

DR. PARISER: Thank you very much, Dr. Abichandani. I wonder if I could please invite all the panelists to please come up to the front, and while they're working their way up here, does anybody have any questions for any of our three speakers so far? Well, while our panelists -- oh, we have Patty. Go ahead.

DR. DICKSON: Hi, Patty Dickson, Harvard, UCLA. So, I think -- Barbara had a great example of a patient who had clinical signs and symptoms that suggested immune tolerance was necessary and PRIA has had many patients like this and I guess the biggest question that we're all asking is, you know, "What about the bulk of the patients?" What about the vast majority of the patients and how do we know we're doing as good of a job as we can and is there -- this industry have any, and I guess for Rekha, for you -- has this -- has there been any discussion about, you know -- can we do better and would removing these antibodies or treating these antibodies, preventing these antibodies -- be a way to make these therapies more effective?

DR. ABICHANDANI: Thanks, Patty. I think the biggest challenge is knowing when to start conducting these clinical trials, you know -- in terms of prophylactically. I think that some diseases -- when it's very that a big

population of will develop adverse -- and adverse impact, I think it's probably clearer. But, doing it prophylactically without knowing -- you know, whether it's going to have a beneficial impact or not, I think it's a little bit harder. So, we are very interested in hearing the outcome of this workshop to help us guide further development. I think that the other question is -- you know -- when do you do it before you -- the big question is, before you demonstrate clinical benefit, should these trials be conducted?

DR. PARISER: Thank you. All right, if I could please have our panelists who have now joined us, please introduce themselves, if they haven't already been introduced -- so, if I could just please start at the far end -- Dr. Burton has already been introduced.

DR. BLUESTONE: Yeah, Jeff Bluestone, UCSF.

DR. TANPAIBOON: Pranoot Tanpaiboon. I'm a geneticist from Children's National Health System in D.C., representing clinicians who treat patients with LSD nominated by NORD.

DR. HOGAN: Melissa Hogan. I'm a parent to a child with MPS 2 or Hunter's Syndrome and do a lot of writing and speaking and I'm an attorney by practice.

DR. WHITLEY: Chet Whitley, University of Minnesota and Chairman of the World Symposium.

DR. WANG: I'm Yow-Ming Wang. I am the Biologic's Team Leader in the Office of Clinical Pharmacology working with the inborn area product region.

DR. KISHNANI: Priya Kishnani. Clinical and biochemical geneticist at Duke University with an interest in lysosoma [unintelligible] Diseases.

DR. ABICHANDANI: Rekha Abichandani, industry representative nominated by Bio.

DR. ROSENBERG: Amy Rosenberg, Division of Therapeutic Proteins, FDA.

DR. TURKA: Larry Turka. I'm an immunologist at Massachusetts General Hospital and Harvard Medical School.

DR. MULDOWNEY: And I'm Laurie Muldowney. I'm a Clinical Reviewer in the Division of Gastroenterology and Inborn Error Products with the FDA.

DR. PARISER: Okay, thank you everyone. If you don't mind, I'm going to stand back here because I wanted to make sure I could see everybody. So if the panelists -- if you have something to say and you haven't caught our eye, if you could just turn your name card on the side and we'll try to call on you. So the panel discussion questions -- well, they're not questions. They're discussion topics and we put some of these up here. You can see them projected on the screen. We had four major areas we wanted to discuss, but

it's not limited to this. As we said before, we'd really be very interested in hearing what people have to say.

So we heard three very interesting perspectives earlier and thank you very much to our speakers for starting us off and I guess what is really coming out here is, well, a number of things we'll discuss further, but drug development is certainly a continuum. We try to get our information in the premarket here but it goes on after approval and we never get all of the information that we want and we need to keep learning as we go along and I think that was very well illustrated and particularly by the patient case presentation because it always does come back to the individual patient and trying to get the information into the hands of the training physician.

So, if we could just please start at the first topic. Discussing the impact of the antidrug anti-bodies on the clinical outcomes and patients and maybe I could ask the clinicians if they wouldn't mind starting us off and maybe giving us their perspectives from the clinic. Priya, do you want to start?

DR. KISHNANI: Yeah, I could actually. I think one of the challenges that we face as clinicians is sometimes these anti-bodies are done as part of clinical trials and they don't really make it into the clinical,

real-world setting. So, for instance, the pathogens set is not very clear and even if it is clear, then it's not really clear how often to collect it, where to send it, and the reporting structure and what the meeting of these anti-bodies are, so I actually faced this challenge initially in working with the Pompe.

I was fortunate to have a lot of partnership with the group at Genzyme, Sue Richards is in the audience here, where we started to recognize the importance of doing this in the real-world setting, so to say, and trying to educate ourselves and our colleagues in this particular arena. Because I think if we don't look, we don't see. And then it's what came first: the chicken or the egg? Was it advanced disease and so there's no response to therapy or why do we accept stabilization as adequate enough for therapy? Is it that the presence of these anti-bodies early on is really impacting, you know, at this patients who are already at the cusp and then getting to a point of irreversible damage or do we not have enough biomarkers or appropriate clinical end points? I think it's just wide open and it's really wonderful that industries here -- to make this a part, I think it's a part of post marketing or education for us treating clinicians to better understand this.

DR. PARISER: No? Yes, please.

DR. WHITLEY: I've got a couple slides. Is it possible to pop those up? Yeah. If you could run them real quick, there's many -- I'll just say -- advanced slide. Oh, you did already. Okay. There we go. There you go. All right. Well, the only point of this slide is to highlight the Lysosomal Disease Network and you can sign up for the meeting I refer to there in a moment, but --

[laughter]

DR. WHITLEY: -- this is it right here. Those are lysosomes expressing iduronidase in a mouse. There's so much Iduronidase in the lysosomes that they pop up as bright red lights with this anti-bodies' stain, but this is actually in a normal mouse. And there's a normal amount of enzymes that's not staining in the background there. But if you look at how we're dealing with enzymes in the laboratory, and I want to highlight this one last row here. Many people are exploring the idea of giving very large doses of enzyme as potentially a way of accessing the central nervous system and this is -- what I'm referring to is intravenous enzyme. These are the studies that have done that. And the study I'm going to mention here, we're getting actually 20-fold the normal amount of enzyme intravenously to mice with iduronidase deficiency or

Hurler's Syndrome.

And if you do that -- there we go. You see what you expect. Increased enzyme activity in many of the tissues, but notably somewhat in the brain, in the lower right-hand corner and then the middle bottom. And furthermore, if you look at the storage material, glycosamine or glycan or GAG, one also sees correction in most of the tissues, including in the brain. A lower, right-hand corner you see the cerebellum has actually got reduced GAG even though we acknowledged the fact that there is a blood-brain barrier of sorts. Also, once sees disappearance of the pathological glycosamine -- or, I'm sorry, gangliocydes. The untreated mouse has got a pink-staining gagliocyde in the brain and on the right side, that's been disappeared or diminished in the hippocampus after intravenous enzyme replacement therapy. And I think this is the way the world is going to go. Notably there's actually a clinical outcome, if you will, with these same mice with Hurler's Syndrome having improved learning after several doses of intravenous drug. There's an impact on the brain.

So having made that arguments, what happens when you give a very high dose of enzyme? Well, you get a very high titer of anti-body. So on the left you'll see a very

dramatic rise in your lyso test showing that there's a huge amount of anti-body made against the drug you're giving intravenously and yet there's an effect on the brain. So we don't understand the impact of enzymes in terms of -- I'm sorry, of anti-bodies in terms of their pathophysiology and how they're really effecting the disorder. I want to point out that patients appreciate this as well as clinicians. And this is our NINDS NIDDK-funded Lysosomal Disease Network. There are 20-some patient advocate groups that participate in our research, and here actually Steve Holland represents the National MPS Society. He is present at the meeting. He can speak to some of the impact on patients.

But I want to mention one additional example. We do send anti-body levels routinely on these patients and there are real difficulties as Dr. Burton pointed out and Dr. Kishnani pointed out in how to interpret the results, when to get the results, and how those specimens are actually handled. In fact, the case that Dr. Utz is going to speak about in the second session is regarding a patient who we'll refer to as P.M. Well, when P.M.'s family heard that we were monitoring anti-bodies, so did all the other families in our clinic. And they became very concerned. And so therefore we monitor more intensely those same parameters on all the patients. In fact, in this one

particular patients where anti-bodies were present, it wasn't really the anti-bodies that were the problem but the fact that there was a specific neutralizing anti-body that was having an impact, so we had to go to some special efforts. I think Dr. Utz will probably comment on that.

But when we look at the other families, we started getting results back and we ran into this kind of problem where a specimen came back and it was unclear as to who the patient was. The two that went out from our hospital is actually labeled M.P., but in actual fact, it was from a patient whose initials are P.M., depending on if you're using the first name or the last name. And one of the things we've noted that when a study, rather when an anti-body is sent as a registry study is that the laboratory that's doing the analysis might be adhereing to the CLEA certification requirements, but it is not fitting into the usual way in which specimens and reports are handled by the hospital. These are often done at no charge which makes it actually quite difficult for the hospital to send out specimens. That doesn't fit into usual clinical practices.

And furthermore, when results come back, they come back to the ordering physicians which is not the way results come back for any other clinical laboratory test. They

always go to the hospital or the sending lab, they're entered into the medical record, and once we take a test out of that usual clinical loop, results get lost, the dandification of this patient specimen becomes at risk, and a lot of problems occur.

So my comment is that I think one of the huge gaps is not only understanding the rules of anti-bodies, but having more specific tests like neutralizing anti-bodies and fitting those into clinical practice. More and more pharma companies are using such tests or moving them out into commercial laboratories and that's been a huge improvement. I think when that happens more and more, we'll find that this kind of improvement in reporting results and being able to interpret will happen. So I want to invite you to the 11th meeting which will be in Orlando and if you want to find out about -- and this will be a hot topic, by the way, at that meeting in Orlando in February of 2015.

DR. PARISER: Yeah. Thank you for bringing up that also very practical aspect to treating the patient. So, our other panelists? Yes?

DR. WANG: Speaking of gaps, I noticed one particular element that the Office of Clinical Pharmacology Colleagues very much invested our energy into. That is the exposure response, monitoring of the drug concentration.

That particular element is not very much mentioned in this context of rare diseases and Lysosomal Disorder treatment. So what I am thinking, in response to the earlier question about why are we seeing a concentration of the drug going on, but, you know, the effect of the drug makes no difference, right? All these are really, to me, is a gap where clinical pharmacology can play a role. We -- I'm thinking for -- if you are lacking the neutralizing anti-body ASA but you have a drug ASA which you can capture the active drug concentration, maybe. That is a surrogate. A substitute for the neutralizing anti-body as in the absence of it, of course.

So but in terms of drug ASA, if you think about other field, the gastroenterology field, they're looking at the drug concentration at the guide to whether or not we rise up or increase -- does escalate or we stay with the same dose. So this is some area -- an area that we can start thinking about, probably, from these in the context.

DR. TANPAIBOON: So, as the clinician, I agree with you. You know, talking about it where they have the anti-body and how accessible that would be. The other thing is although some companies do offer the anti-body for free for the patients, but a turnaround time takes ridiculously long. Sometimes it takes a year to get a result. And we

might accept that some of the patients actually did get the treatment not with geneticists but with other physicians. For example, [unintelligible] some patients treat with nephrologists, so I don't know how much they order their patients. Sometimes it's just do the enzyme replacement therapy without more or the other organs might be affected by the disease as well. So I know from the rich history they have, like, a recommendation how often we need to monitor the anti-body, but again we can't do that often as the recommended.

DR. BLUESTONE: So I guess I'd just start out with just sort of a general comment. I'm a basic immunologist and a Ph.D. scientist so I don't work with the patients directly, but start with sort of fundamental principles for me and the fundamental principle being that the anti-body is a read out of an immune response against the protein that's being injected and a decision needs to be made. Is that a good or a bad thing and in spite of what Dr. Rosenberg said about the lack of clarity sometimes about whether it might enhance the P.K. of the product in general, making an immune response is not likely to be a good thing against the protein and the lack of correlation with the anti-body titers could be affinity, it could be other epitope differences, but fundamentally there's still likely to be an

immune response. So, I study type I diabetes. The auto-antibodies there aren't particularly damaging except in a very few patients who can get stiff man syndrome, but for the most part the anti-bodies against insulin are not damaging, but it is an auto-immune response that destroys the pancreas.

So there are a lot of other things that happen when you have an immune response besides just making auto-anti-bodies. You are making T-cells that can recognize the antigen that might make cytokine such as TNF or interferon that might themselves cause damage to tissues. It can, in some cases, even make it inside cytolitic T-cells that can destroy where the enzyme might be.

So I think you have to start out with the first principle: Is it a good or bad thing to make an immune response against the protein that's being injected in these patients? My own feeling in general is that's likely overall not to be a good thing and therefore it's worth thinking about ways of first understanding why you're getting it. Is the nature of the protein responsible, the quality of the protein, the contaminations in the protein, the glycosolations of the protein, et cetera. But if you're going to get an immune response and I think it behooves the community to think hard about how to avoid that because in

general I would believe if you can avoid an immune response against a protein that you don't want an immune response against, then it's worth looking into that and so I would encourage the group to think about more generally how does one avoid getting an immune response because I think in the end this is not likely to be a good thing for the overwhelming majority of the patients.

DR. PARISER: Priya?

DR. KISHNANI: I couldn't agree more with that comment over there because even in the infantile Pompe population -- Chet, this is to your point of the slide that you showed. Sometimes these patients continue to show clinical benefit until there's a certain threshold point. You know, when you're really not getting the therapeutic protein to the target site and until that point, the patient continues to look good and you may stop doing anti-body titers at that point and then after that you may see a clinical decline. That's exactly what we saw, even in the sickest infantile Pompe patients that Dr. Rosenberg and others have talked about. So I think that it has to be a systematic collection of this information over time before we, you know, draw conclusions. Whether it's really doing something or not doing something. And I think the second part is that the kind of end points that we use or the kind

of biomarkers we use, we've chosen to believe that stabilization is a good thing. But we don't know whether it really is a good thing. I mean if we really looked long and hard enough and you looked at two cohorts, one with an immune response and one without, we may start seeing differences even in what we're calling stabilization versus not. This is just an appeal here.

DR. PARISER: Let me look down the far end. Oh, we have a question? Could you please come to the microphone?

MR. RICHARD:

Yeah, my name's Charley Richard of Oxyrane. I have a question for the panel kind of following up on this idea. There's increasing evidence from animal studies and human patients, especially in Pompe disease that immune tolerance induction is much more successful in treatment naïve patients and that once patients have sustained high titer anti-bodies can be done but it's much more difficult and so I'm -- maybe this is a question for Priya who's I'm sure thought a lot about this. Are we on the cusp or where do we need to go to be thinking about immune tolerance induction in treatment-naïve ERT patients and looking at the risk benefits of immunosuppression with the problems of trying to suppress anti-bodies once they've already formed?

DR. KISHNANI: Charley, thank you so much for that comment and that question. We are going to be doing a session in the afternoon and you're right. I have thought and many in the field have thought about this and I think if we did have an ideal protocol, you know, where we looked at the risk-benefit ratios and it went more in the bucket of it's acceptable to do if it's short and it's safe. I truly believe that this may be the way that we have to start thinking about this but we can get back to this in the afternoon session. Thank you.

DR. PARISER: And Dr. Muldowney, let me put you on the spot a little bit here but can you give the FDA Review Division perspective on some of what we're discussing here?

DR. MULDOWNEY: Sure. I can -- and I'm a clinical reviewer in the Division of Inborn Error Products, so I'm not a clinical expert in these conditions, but I can sort of speak to the -- some of the challenges from the review perspectives and some of it has already been brought up that in these small disease populations where the clinical manifestations of the disease maybe very slowly progressive and heterogeneous. It's very difficult to assess the impact of anti-drug antibodies on the disease course when we receive a marketing application to review. The guidance, I think, is very helpful and hopefully we'll begin to see

really standardized information coming through. But adding to the challenges that exist inherent in the disease populations to begin with, historically it's been a challenge because we haven't always received all of the information with the initial applications, so the amount of immunogenicity data that's been available has varied.

So sometimes we might have a validated binding assay, but we might not have titer information. Or we might not have information neutralizing antibody titer information, which make it very difficult to assess. And we've talked a little bit about CRIM status and genetic mutations as well, and often that information is not available at the time that we're reviewing an application as well. And where in the past have been asking for those things as post marketing commitments, post marketing requirements. But hopefully we'll start to see more of that information at the time that we're reviewing the application and I think that that would be a benefit. DR. PARISER: Okay. Well we have a -- oh, Mrs. Hogan, would you like to say something?

DR. HOGAN: And I just wanted to comment that in especially the slower-progressing or relatively slower-progressing diseases, such as MPS2, I think we're really starting to see just the tip of the iceberg. Because until most recently, if you're talking in terms of families having

any understanding of whether to ask about antibodies or ask for antibody testing and especially more local physicians, as opposed to some of the more expert physicians in MPS and LSDs here, knowing anything about antibodies or to even monitor those, this is really just the tip of the iceberg to understanding the impact I think that has in the slower-progressing diseases.

DR. PARISER: So, Amy.

DR. ROSENBERG: So to get to Jeff's point where that antibodies in general, except in those very few exceptions which are unpredictable and uncontrolled negatively effect the product activity starting with that and going to the fact that these are small, you know, small patient populations, you may not have a lot of data. We really have to use, I think, all of the evidence available to us. And some of that comes from good animal models, such as the MPS1 dog. Which, I think, the studies that Dr. Dickson did really brilliantly showed that if you're tolerant, you get increased amounts of enzyme activity in critical target tissues. You're giving the patient the best chance for success and the best chance for optimization of the therapy. Without it, as you saw from that work, the non-tolerant dogs did not achieve significant -- a really significant amount of enzyme activity in critical target

tissues. We've got to use all of the data available to us in moving forward. And that will also include being able to assess patients for their antibody status, not just in a clinical trial, but as it's needed. So that is something, I think, that's really going to be very critical for FDA to ponder; how to be able to get that testing done when it's needed.

DR. PARISER: I think I've heard from several of our speakers then of what is going in, I think, in a very similar direction, is, these are serious diseases and we want to get into the clinic as fast as we can and we want to get patient access to these drugs as fast as we can. And we have to balance that also with the need to get information so that we can inform physicians, give them the tools they need to ultimately treat the patient. I mean, we heard from Dr. Burton's -- she's talking about how individualized these approaches have to become and how you're often on the ground using whatever things you have available to you in these individual approaches.

So I think that this does bring up some things that we need to discuss about what is it we should be doing in all cases, in some cases, do we not know what -- do we need pre-market? This is something that is going to be a continuum as we continue to go, how do we best capture the

information? And as Dr. Whitley brought up, these are, kind of, off the grid type testing. So it's important to capture this, and how do we get best available information? So would someone like to comment about what are the things that we are critical during pre-clinical testing, what are the things that we need to bring forward or do we not have that information? Dr. Whitley --

FEMALE SPEAKER: Sorry, I have a question here before we change subjects.

DR. PARISER: Yes, please go ahead.

FEMALE SPEAKER: Just to talk about the clinical testing. So as Dr. Rosenberg said this morning for Vimizim -- I'm from BioMarin, Becky Shwardhart [spelled phonetically] -- we didn't find any discernable impact of patients that had higher antibody titers and less of a P.D. or efficacy effect. So in that case, we've had physicians as our drug as gone to commercially available, they have requested for the antibody data. But we can't tell them how to interpret that data because we have not seen an increase -- or a decrease in the treatment effect as the titers increase. So in that case, you know, what would be the approach? Would the approach be to take -- to collect the antibody samples and then only test them if you see a decrease in treatment effect in those patients? I mean, to

do this continuous testing is labor intensive and very expensive. And who would be paying for that? Is that a post-marketing commitment for the company to continue to do, even though we don't see a decrease treatment effect?

DR. WHITLEY: That testing pales in the context of the cost of the drug. If I can say, I think it's absolutely essential we think about what testing is appropriate as a clinician, I'm going to say, we're finding out that the antibody titers are, if not useless, they're not informative. But what does seem to be, in our experience, and there's obviously a lot more that needs to be learned, is a test for neutralizing antibody. And that should be, theoretically, very cheap. And a drug manufacturer could either do that itself because it has the drug, or provide that test -- provide that drug, rather -- to a commercial laboratory. Furthermore, I think clear regulations require a relatively timely submission of results to the requesting physician or institution. And they also always have an interpretation, that is one of the requirements of a laboratory test. If all you know is, we don't know how important this is, you say that, but you give a number.

[talking simultaneously]

FEMALE SPEAKER: Right. So my question is not really about the --

DR. WHITLEY: I think there are very clear recommendations --

FEMALE SPEAKER: -- the price tag that goes along with it. But if we've already done extensive clinical trials and we don't see a correlation between higher titers and a reduced treatment effect, how is a physician going to interpret that information? And how do we continue to interpret that information?

DR. WHITLEY: Well that's really going to be up to clinicians. And believe me, those of us who have to deal with these patients, we want data and we can interpret data. And, you know, we can do it as a group in meetings like this. Or we do it in a one-to-one basis with the patient in front of them. But if we have a number -- I have a one Gaucher patient who every time insists upon seeing his antibody titers. And, in fact, because he started on a very, very low dose enzyme and has increased, he actually does look at some of the other biomarkers. And then he himself is very comfortable and accurate in interrupting those. So I think people want number --

FEMALE SPEAKER: How is he accurate in -- sorry. So we've done extensive clinical trials to look at this in many people. So doing one off, you know, trying to determine from one patient what the impact of that antibody

is, is impossible. So yeah, Priya?

DR. BLUESTONE: Can I make a comment on the neutralizing antibodies, just to comment?

DR. PARISER: Sure.

DR. BLUESTONE: So I think we're all hearing sort of the same thing. Which is, "We definitely need to learn more." But I would be a little bit cautious that the solution's going to be in the neutralizing antibodies titer. As you saw in Dr. Rosenberg's slides, a lot of the changes that took place in the dogs had to do with where the molecule went -- liver as the major depot. And that doesn't necessarily require neutralizing antibodies. It just requires an antibody that allows the protein to be sucked-up into a F.C. receptor baring tissue. So I think we need to learn a lot more about this. I would disagree that we should just assume, because so far we're not smart enough to figure out how the antibody titer correlates, that we shouldn't do it. I think we should. I think looking at neutralizing antibodies is important, but we should be well aware the fact that antibodies can do a number of things. And they don't have to be neutralizing to have an effect.

DR. KISHNANI: I could make one more comment, I appreciate your comment that, you know, that you've done it in a large clinical trial. But you've only done it for z

number of weeks, and x number of population that met in crucial criteria for your trial. It's still not the real world setting. And I will give you an example of what we did in the late onset trials for Pompe disease, and we have a representative from Genzyme; the clinical trial data did not show an impact -- a negative impact -- of antibodies in late onset Pompe disease. And yet, even the data for Amy Rosenberg from that paper says that there was neutralizing activity, and in fact, those patients appear to look good.

But that was 78 week data. Now when we look at the real world and we have published on that, there are a subset of patients that do develop high titers. It takes a way longer period of time for us to see an impact, because I don't think we have ideal endpoints at this point. And so we were still able to show some changes in six minute, etc., which did correlate with the high titer. So I think that we are still in a stage of infancy, we've only touched the tip of the iceberg with the very sick infantile CRIM-negative patients. And I think we've got to do this collectively as a group for us to continue to learn. We may not have answers today, but we shouldn't kick ourselves ten years or five years later and say, "Oh we did not collect it, so we don't know what do with this information."

FEMALE SPEAKER: So, yeah. And then a question

would be, do you just bank the samples and you test them when you see a reduced treatment effect in that patient?

DR. WHITLEY: My answer is, "No" to that one. I mean, it takes a very long time to see the treatment effect. I mean, if you've done a clinical trial, you've already dealt with that. Probably had a very, very hard time finding meaningful outcomes. Some of those are only going to occur later on as Priya is alluding to. So I think what you want to do is get the data out there in real time and learn as it's happening. The alternative is for the FDA to say, "These drugs are not really as efficacious, we want all that data upfront." So if that data and that very, very extensive testing looking at uptake of enzyme assays, looking at neutralizing antibody testing, if you're not going to produce that upfront, then there's an obligation to do it in real time afterwards.

DR. PARISER: Another question, please?

MS. RICHARDS: Yeah, this is Sue Richards from Genzyme. I got a comment and also a question for the panel. So with respect to the antibody testing, at least with regard to the Genzyme replacement enzymes, I mean, we do for all potential patients, the paradigm that's been shown. So, you know, the binding two neutral antibodies uptake as well as inhibition of enzyme activity, plus drug-specific IGE,

they're done in assays that are validated following FDA guidance documents and a CLEA certified laboratory.

So this testing is available to all patients that are on replacement enzymes, not only patients in the clinical trials or enrolled in the registry, but any patient from a treating physician that submits a sample is tested and there is communication between the GPE officer as well as the CLEA lab director is available for consult to help an interpretation. So I know that that's our paradigm when we do. I can't comment on other companies, but that's what we follow. In listening to the conversation, I mean, one thing that I'm hearing as a gap is, how this information gets disubnated across treating physicians.

Do we need a component of this that's a more thorough, in depth education for treating physicians of what the data are collectively, how to potentially interpret them, and where to go with this? Because that's a space that seems to be a gap. We go to the specialist, but the specialist is not always the broader treatment group and it's really a team that's treating these patients. And it's the team that needs the information. And that's a place where, perhaps, there is a gap that needs to be a much broader education in this particular space.

So that's the basis of my question. I wanted to,

kind of, get some feedback. What does the panel think in terms of an educational aspect here, and working broadly as a team with this information? Because collectively, there actually is a lot of information, but may not be as broadly disseminated as it needs to be for patient treatment.

DR. BURTON: I'd say a huge "Yes" to that. I think, I mean, I loved hearing what you just said because I actually wasn't aware that the assays were available to patients not in the registry. So that's good information and, I mean, all our patients are in the registries. But, you know, it's good to know that that's the case. And I think that would be hugely important, that kind of educational effort. Because I do think that doctors don't really know, there's no guidelines -- how often should they send it, should they just send it if a patient has a reaction, should they send it routinely? You know, what do the results mean? You know, I really think that would be so helpful and so important.

DR. TANPAIBOON: So as I already said, there's some physicians who treat patients with LSD did not [unintelligible]. And some of them, although they are geneticists, but they don't have a main interest in LSD. It's so hard to our LSD patients to come to the big center, the center that has LSD experts and do the treatments.

Because, you know, due to incretion every week, every two weeks, every month, it's hard for them to come. So why are things a weak point? Because my interest is biochemical geneticism -- biochemical genetics and LSD as well. So we are talk about how to treat patients with LSD, how to monitor patients with LSD in LSD meeting. Or some small meeting. But we rarely talk about LSD or the treatment or the effect of treatment in the big genetics community, just very rare to find that type of topic, or for example, as I am M.D., there are talks about small molecule treatment. But, you know, some of the talks in that meeting actually to treat patients with LSD.

DR. PARISER: Dr. Turka.

DR. TURKA: Well obviously to say that I think that there's, you know, a potential opportunity that might exist out there. There's -- sounds like there's a lot of data or potentially a lot of data. And as I think about the community of people, I'm a physician but I study really transplantation auto-immunity. But there's a lot of relevant areas and other immunological disciplines and minds that could be brought to bear upon the state asset. In the context of allergy and antibody responses and the context of the people who study vaccinations in a deliberate effort to elicit antibody responses, people who study auto immunity or

transplantation would be potential interested and be able to, maybe, contribute to the dialog if they -- if the data was out there in a anonymized form that scientists and physicians could look at and see what they can -- what ideas that that might generate in their minds.

So I think that there's a whole community of people out there who would be both -- who'd be interested in looking at the kind of data that might be available and might be able to contribute to this dialog. And as well, those kinds of individuals that might become interested in a more meaningful way in looking at these patients and trying to devise better strategies. So it's both a way to think about getting more knowledge base, and even recruiting other minds into the field.

DR. HOGAN: And when we were discussing the aspect of sharing among providers, for patients, I think, in a lot of the circumstances, especially as she said, "Not at the big LSD centers." It's the families who are actually doing that and sharing that information among their providers. And so I think, only by knowing the information and being able to look, you know, honestly, this is really a trending issue. Because it's not a one point and time. So the families are having these conversations and comparing and looking at their antibody numbers themselves. And so they

need that data in order to do that.

DR. ABICHANDANI: I had a question -- this is Rekha Abichandani, industry representative. Could the panel comment on whether CRIM assays should be systematically developed for all enzyme replacement therapies? I think there was some variability in how companies are approaching this. But, you know, should they all be developed prospectively for all LSDs or should it be a case-by-case approach?

DR. KISHNANI: Rekha, this is Priya. Maybe I can answer, I want to first make one comment of what we even call a CRIM assay. Because a CRIM assay can vary from person, to lab, to company. And so I think we first need to have a standardized way of understanding a CRIM assay. Because, I think, we can do a lot of disservice if we put up a CRIM assay which is not reflecting, you know, what it's supposed to do. And we've had this experience with, again, Pompe as an example; where it's done in certain labs, we're trying to rush, we're trying to do it now with the blood based assay. All that is wonderful, but at the end of the day, I think you do need some correlation. The amount of protein that's put in some standardized way. And also correlated with mutations and then also with your antibody information. So I think, yes. It's important to do it.

But it shouldn't be the stand all, be all and say, "I did a CRIM assay, it's CRIM positive, so we've moved on." I think that it's beyond -- I think there's a spectrum of CRIM. Like we say that there's spectrum of disease. And this is to say some of the strongly positive -- CRIM positive -- adults in some of these diseases develop high antibody titers. And so I think that becomes part of that whole discussion thread.

DR. PARISER: You have another question? Go ahead.

FEMALE SPEAKER: Yes. Susan Kirshner [spelled phonetically], FDA. So I wanted to talk a little bit about the anti-drug antibody assays. And right now there's still relatively blunt tools that look either generally at binding, or more specifically at the ability to inhibit in a usually bioassay. So I would think that the inability to see a correlation isn't a call to stop testing. But a call to do more in-depth testing and to develop less blunt tools. There are even still fairly blunt tools, such as isotyping and some epitope mapping that aren't systematically done. So I don't think the idea is to quit testing, but to now, if we're not seeing correlations, look more specifically at the antibody populations and how they may really be playing into the disease.

The other thing I wanted to say is I think Jeff brought up a very good point. What is the impact of having a chronic antibody response against an antigen overall in the patient. Not just necessarily in the specific disease progression, but in general? You know, how do people respond when they're constantly in that kind of pro-inflammatory state, and is that really a desirable state to be in?

FEMALE SPEAKER: Okay. Hi. I'm Prishy Romand [spelled phonetically] from Celgene, a foreigner to the R.P. world. So I see that we're trying to classify neutralizing antibodies as either optic neutralizing or activity neutralizing. And it looks like a sustained PK might be helpful only in the case of optic neutralizing from Amy's presentation. Do you see patients who are exclusively optic neutralizing and activity neutralizing or do you usually see in clinically practice that patients have a little bit of both? And does this change over time within the same patient? And secondly, if you see exclusively optic neutralizing, does it result in a change in PK which all seems to be suggested by the chaperone story. Do you think it's because of FCR mediated uptake and non-neutralized active site? Sorry. That question was long.

DR. ROSENBERG: Yeah, so it's complex and Priya

can, I think, help me here. There are some patients who only -- exclusively make uptake neutralizing and those who exclusively make catalytic targeted antibodies. But there's also significant overlap, patients who make both kinds of antibodies. And the, you know, the question of; when do up take neutralizing antibodies lead to a better profile, versus really neutralizing the efficacy or the activity of the enzyme is a good one and seems that if the antibody titer itself is continually rising, then that tends more towards diminishing the efficacy. So I think it's a, sort of, narrow range at which you get the favorable effect and again, it may be a metastable state. You know, if you got -- a patient gets an intermittent infection, maybe that's going to boost up the response.

So, you know, again, I think it's an excellent strategy to think of for formulating these enzyme replacement therapies. Can you use this to formulate an enzyme replacement therapy that's longer-acting and more efficacious because it's protected from, you know, various means of mis-targeting and uptake. And, you know, to me that's what sings out from this. We don't know, for instance, with the Morceo [spelled phonetically] patients whether if they were tolerized they would do much better. We don't have the answer to that. Jeff's question also

about finely mapping the epitopes specificity of those uptake and neutralizing antibodies is also a good one because in cytokine literature the specificity -- the epitope specificity matters in terms of the potential for the antibody to act as a carrier and not to act to diminish activities. Is that correct, Jeff?

DR. BLUESTONE: Yeah, I think so. And in fact, it would open up a whole area of potential research, where if there are dominant areas that could be, in fact altered without losing efficacy, but might lose imaginicity that would be a really important area to know about, I would think, for the industry.

FEMALE SPEAKER: One part of the question was; so the F.C. mediated uptake. Does that result in activity of the enzyme? I'm not familiar with the pathway the enzyme has to take to be active. So if there's FCRN mediated uptake of --

DR. ROSENBERG: The FCRN, yeah. What happens with that is the activity ends up being blocked. It ends up being presented in a class two MHC presenting pathway and, if anything, it sort of, perpetuates immune responses.

DR. KISHNANI: Okay. If I could make one comment here, Priya, is you asked about. I don't think these assays, we have patients with very high antibody titers and

zero neutralizing as this is an in vitro-assay. And we have some with lower titers, or not as high titers and with positive neutralizing activity. So I think to the point of what Jeff made, I think the presence of very high titers or the presence of persistent of titers of a period of time is, for me, what matters as a clinician. I look at the neutralizing activity, but the absence of it does not tell me it's not meaningful. I don't think we have assays that are really completely well developed at this point, in my opinion. These are in vitro first-step assays and not always giving us the information that we're looking for.

And then there are other pathways, you know, citocondrolys [spelled phonetically], IL-6, which we don't even look at in terms of, "What are these antibodies doing?" And I think that's where this whole field, we have to start thinking beyond just neutralizing activity. But also what's the role of these T-cells and what's happening when you have an immune response and cascade that set up. I'm not an immunologist, but --

DR. ROSENBERG: Sorry. Yeah, the other thing we're not looking at is the infinity of these antibodies. And that may make a big difference in terms of the ability of the end sum to dissociate and bind to target tissue. You know, it may be that a relatively lower affinity antibody,

though neutralizing is a good thing, whereas the high affinity ones, you know, it doesn't get to dissociate. So I think that's another thing we really have to look at, is antibody affinity.

DR. WANG: Yeah, speaking of all these questions and all these hypothesis, I thought that there is opportunity where we can actually integrate a lot of available data to address these questions. One example was what Amy presented earlier about the dog data. And we have tons of animal data. I know that they're not necessarily reflective of the human antibody information. But knowing the system in the preclinical system, knowing how the affinity effects the binding, how the uptake inhibitory antibody effects the drug effect, are those system-type of questions probably could be addressed by building an animal model and having a canatic and understanding of it so that we can later on use more sparse human data to, you know, replace certain variables in that system. And not being a physician, I get to dream-up all these different types of computational modeling that could possibly help build a good tool for the physicians to, you know, pave the way for the future.

FEMALE SPEAKER: Well obviously, I agree with you that using animal models is really useful. I mean, there

were certain -- many things we learned from the tissue data that we could never have learned from human subjects. And I'll give you one example that hasn't been brought up yet. And that is; we looked at intrathecal enzyme replacement therapy given in the K9 model, which is a MPS1 model and looking at enzyme therapy given into the cerebral spinal fluid and we found that in dogs that had a high antibody titer against the enzymes, we had less reduction of sub-straight in the brain tissue. However, if you look at the CSF glycogenic gylcans, they were reduced equally in the dogs with and without antibody titers. So you can imagine in a clinical situation, you're not going to be able to tell that you're having a slightly decreased effect in the MPS patients. Although, we can see it at the tissue level.

I wanted to make a quick comment on an epitopes as was brought up before. I don't know about for the enzymes, but I know that for iduronidase, the epitopes were mapped in a paper that came out about a decade ago in Lancet. And it was Rebecca Kakavanos with Emil Kakkis and the epitopes were all over the protein. And in terms of blocking uptake, you know, the uptake of these enzymes is through mannose-6-phosphate, which are going to be located in a variety of places across --

[off the record]

Public Q & A

DR. PARISER: Okay, well I think we can probably start up again. So, this is the, we call it the public question period. But it's really the floor is open if anybody has any questions that they would like to ask. If there's anybody who would like to start us off, please work your way to the microphone. If not I think we have a few questions that we'd like to ask as well. So, yes, well give it a couple of --

[talking simultaneously]

FEMALE SPEAKER: Some of our panel are not here.

DR. PARISER: Yes, people are still filtering in; we'll give it another minute or two. Yeah, if the panelists could please, if they wouldn't mind, please come back up to the front for additional questions.

[inaudible commentary]

DR. PARISER: And here come a couple more.

[inaudible commentary]

DR. PARISER: Yep, yep, we're ready.

DR. KISHNANI: So, good morning. I hope everyone had a good break. And, this next 60 minutes is really for

public questions and answers and this is really intended to be very interactive. To try and address some of the outstanding issues we have. So if there are any questions either from the phone line, is that available? Or, as well -- no. So it's just the audience here. I guess we'll give people a couple of minutes to settle in but for those who are here if there are any questions or comments that you would like to make, that would be terrific.

DR. PARISER: Okay, well, I have a question. So, I mean, I think this is -- it came up in this morning's discussion. I mean clearly we have a need and there's a strong desire for more information and better information and what we're particularly looking for is trying to put that information in the hands of the clinicians who will be treating the patients and by extension into the hands of the patients and parents. To ultimately get the best care that we can for the patients. So, a question then for clinicians and also parents in the audience; how can we better arm you with the information that you need? What are the things specifically that we're looking for? We've heard some of this touched on this morning, but again, this will cross into both the pre and post market periods.

So do any of the clinicians want to comment?

DR. KISHNANI: Pranoot, do you want to take a

first stab and then I can ask Dr. Muenzer [spelled phonetically] who I see in the audience?

DR. TANPAIBOON: So, um the question was how we would be helped with the auto [unintelligible] and everything. I think for --

FEMALE SPEAKER: Could you speak up, just a little louder? Thank you.

DR. TANPAIBOON: Can you repeat the question?

DR. KISHNANI: She wants you to repeat the question, Pranoot does.

DR. PARISER: Oh, okay. So, then how can we better, better arm the treating physician with the information they need when they are in the clinic and they have the patient in front of them? We've heard that the labels aren't particularly informative, but that is the goal. To try to get the best information to the treating clinician so that they can take the best care of their patients.

DR. TANPAIBOON: I think you need more data, like long time, long term clinical data and compare whether or not what, if antibody can actually have an effect on the treatment or outcome the patients. The last speaker had a talk about it and we don't know for sure if patients don't respond to treatment is because our antibody titers or

because of the disease itself or because of the ERT can't get to the target cells and help the patient to be better.

The other thing is, I think because we have small number of patients it's just hard to get good clinical data and we just need to have more collaboration between other centers and maybe other countries to get more number of patients in terms of getting the better data. We need to get a good guideline. I know that we, there are some guideline available but it's not that new guideline. I think having new guideline, this guideline in the [unintelligible] genetics publication or journal or maybe in the Journal Genetics article, that would be very helpful as well.

DR. PARISER: Joe?

DR. MUENZER: So good morning Priya. I normally don't see her except at meetings, even though we live about 10 miles apart. Joe Muenzer, UNC Chapel Hill. There are lots of issues related to the physician accessing to titers. One is that each disorder has its own assay and therefore comparisons for people across MPS disorders, across Pompe, across Gaucher's, is impossible to do in the current climate. I can't say a titer in Pompe of one to 100,000. What's that equivalent to in an MPS two patient or an MPS six patient. So that's one of the major challenges.

There's no standardization. And I'd be curious what the panel in terms of what the FDA response is. Is that, are these tests equivalent, or are they really very different from disorder to disorder? Since each one is developed by the particular company that's sort of sponsoring that particular drug.

Amy, you want to tackle that?

DR. ROSENBERG: I think I'll pass this to my clinical --

[laughter]

DR. ROSENBERG: I really don't have a lot of insight here.

DR. PARISER: I'm not sure I'm going to be able to provide, I mean I think that that's a challenge for us as well. That the assays are not standardized. And I think the point was brought up earlier about an interpretation of data for the clinicians and how frequently to get drug antibodies and how to interpret those results. And, because it's specific for each disease and each drug, really it has to be specific guidance. And I don't know if -- you know, I'm not sure if it's possible to standardize these assays. You know, of course they're each directed, they're each directed to a different enzyme so there's, it's not possible, I'm not sure if industry can speak to that and

whether there's any way to provide better information or improved information on these.

DR. ROSENBERG: I think Susan Kirshner would like to make a comment, who's written a guidance on assays.

FEMALE SPEAKER: So you're right. The assays aren't standardized and there isn't going to be a good way of standardizing them. What we do is take the data that we get from the clinic trial with the assays and do analyses that are specific for that assay and that, those clinic trial data and the results obtained from that assay. And we try and understand given that assay, whether we can see an impact clinically. So you're not going to be able to look across diseases and say "one to 100,000 was important in MPS so it's going to be important in this." It's just not possible right now. And I think Sue wants to talk about it and she's better than me for that.

MS. RICHARDS: Yeah, Sue Richards. So, the question about whether or not one can compare titers in assays across different drug products for replacement enzymes. I don't think you really can. And the reasons I would say it is, you know these molecules are of different sizes, different complexities. Different amounts of glycosylation. So myozyme, which is a large molecule relative to, you know, serozyme which is half roughly in its

size. You'll have a different density of epitopes between the molecules and that'll end up in and of itself the possibility of having different titers and infinities. So to be able to do that across products probably is not possible. One should however be able to do it with an assay across different studies of a particular drug. Hopefully that's helpful.

DR. MUENZER: That I think is very clear. That's one of the challenges. So therefore, education is so crucial to get people to be aware of that titers in one disorder might be very different in another disorder. The other is this definition of inhibitory antibodies. Or neutralizing antibodies. Neutralizing activity probably done a variety of different ways. And, again, the same challenge that we have with titers. How do you look at the more sensitive sort of neutralizing or inhabitation of uptake which to me is another standard in terms of how to utilize that. And that's again, this issue of lack of really lots of experience with these products and the lack of ability of really published data. There's very little published on antibody titers except for maybe in Pompe and any of the others looking at neutralizing, looking at titers, looking at genotypes. Even when you, because they're so a rare disorders that's really the limitation.

So that's, another issue is the lack of really, as Chad already alluded, and other people alluded to, is the rapid availability, if I ask a titer for some of the MPS disorders or even, it takes a while, it can be months to get those back. And so that's a limitation. If there can be a commercial lab where you actually have those results relatively quickly that may help, it may not help, but at least we have -- and to know what the normal ranges are, that's, what are -- you know, in the clinic trials where the, really the only data is current published. For example I can speak to MPS one and two, those titers tend to be relatively low compared to when we treat the more severe patients.

And the clinic trials always treats the attenuated patients because they need to do things. They need to do a six minute walk or PFTs. We don't treat the severe forms of MPS one and two, for example. We have, in the clinical trials and so all of the data we have from titers. There's a good example from MPS two, the severe form now shows very high titers. Some of those patients who, as we just heard from Dr. Burden, that patient who has a complete gene deletion has a titer of greater than one in a 100,000. Where in the clinical trial titers of one in 50, one in 40, one in 80, you know, the relatively low titers mean very

little.

And so we didn't see any affects. But clearly in the severe forms of MPS one and two we do see, I think, clinically I do see effects from antibodies. The challenge is how to quantify those affects. We have, we're terrible in terms of bio markers. Dr. Burden alluded to urine gags as a bio marker, but that's all we have. We have nothing else. And I think that's, as you can do in animals, we can't take tissue biopsies of heart values or brain or other things to really show what's going on so that's one other limitation.

But just getting the information out to individuals. What Has happened in the current. Publishing more in some of these, that where the industry and the academics probably should publish more in terms of what is happening and just documenting in terms of titers on these populations would really facilitate a better understanding.

There's no question in my mind that these titers really do impact our patients. The question is figuring out which ones should be treated and which ones shouldn't be.

DR. PARISER: Well, and I just have a comment. And I think your comments highlight two significant problems. And one is just the importance for long term data. Because really the management of any individual

patient is going to be based on their own particular trends and clinical course because we can't compare across diseases. And then the other is the issue that I think we'll talk about more this afternoon in terms of clinical trial design given that a lot of the clinical trials for ERT, maybe almost exclusively CRIM positive patients, I think we're not getting a real sense in the clinical stage of the antibodies and titers before it goes to market.

DR. KISHNANI: Larry.

DR. TURKA: I think maybe this partially reiterates a point I made before, but your point about, and I agree completely, is that we're grouping together a long list of diseases, and we saw slides with 20 different diseases, and there's relatively small numbers of patients in each, and to get data out there and to wait for it to be published I think is going to be challenging because it takes a lot of effort for large series, for small series, to get stuff in the literature.

I wonder again if there's not a mechanism to make data available in a sort of different format than a peer review publication. But, you know, just assay data, the kind of information that you are looking for. In a way that, across all diseases, that people can look at to rapidly get that information transmitted to physicians, to

patients and to other scientists that might be interested in it. Short of having it appear in a paper, a journal, there are secure websites, there are a variety of mechanisms that could be employed for data sharing to help accelerate, you know, research in this and knowledge transmission.

DR. KISHNANI: So I'm --

FEMALE SPEAKER: I wonder if that is something that Dr., that NORD could facilitate?

DR. KISHNANI: That was going to be my question. I think we're coming to at least some understanding of the situation. That clinical trials are not giving us the full information. We need systematic, long term follow-up. We don't have a common, we can't use the same assay, but we can use methods across the different disease states. But I think a question I have after your question or comment Dr. Muenzer is, from industry, is there a way of trying to work together, maybe under the umbrella of NORD. I'd love some input and some comments from industry here because we're all grappling with this at this time.

DR. MUENZER: I think the challenge is really getting the data out. And I agree this is a challenge because writing these publications are difficult. They're very few patients, you know. And journals are not that interested in reporting on one or two patients where all

your doing is saying "Here is what happened" a natural history. I think one of the other challenges is how reliable are these titers. You know in other assays we know, plus or minus, here's the normal range.

I don't know for these titers, you know, certainly for families, they see a jump in doubling or quadrupling of a titer, to me which is not a big thing, but for them it's really the end of the world. So I think the other thing to know is the significance of the, what's the significant difference, you know, because going from one, say 40 to 80 to 160 is probably not a big difference, but if you go four-fold more than it is. So that's another challenge in terms of the lack of sort of, clinical experience dealing with titers. At least in the genetics community. I think in other community they might have more comfort level. But currently in the genetics community that's really, looking at titers is something that's not been done historically.

DR. KISHNANI: So Joe, to this comment, and I'm sure that Dr. Rosenberg can comment to this, but I don't think single titers, and you well know, the trends over time and that's once again why we say we should be collecting this information over time. But now if we can comeback for an industry perspective, if Rekha, Sue, Charlie and others from industry, is there a thought process of trying to have

a systematic approach to having this and a method?

DR. ABICHANDANI: So, I think I'd ask Young Chang Que [spelled phonetically] from Shire and Sue, Sue Richards from Genzyme to comment. Right, I know there are, Priya, there are the different formats, right. But all the assays are validated in their own way. I think if you want to compare, I think that there are some projects where some companies are undertaking, I think like Shire and Genzyme, where you know we've had, unless you have a third independent party do them I think it's sometimes kind of hard to do it, right?

DR. KISHNANI: I think one of the other challenges is if you start down a certain, down a development path with a certain assay to switch mid-stream it's also challenging if you're trying to standardize them. Because you're trying to always compare the totality of the evidence with the certain ERT. In the interest of standardization then you change your assay format mid-way then it's kind of hard to assess the totality of the data with that particular ERT. But, perhaps Young Chang and Sue can also comment.

MS. RICHARDS: All right, I'll start the discussion. So, basically, I think now we're at a point where everyone across industry because of the hard work over the last few years, in collaboration with the FDA and the

guidance documents that are available, kind of we're all leaning toward the same or very similar technologies platforms. The assays get validated so early we have to assign sensitivities.

We have to show specificity. And so these assays now are being validated in a very similar fashion. So that being said, they still, however are in different technologies platforms. They use different reagents. And from that point of view, because you could use MSD, you could use and [unintelligible] electomicrotiterplate, you can do [unintelligible] for binding antibodies. It's hard to compare the assays across platforms.

An approach that has been taken in the past successfully, for example, I would point to beta-interferon. I'd also point to when we had the incidents with pure red blood cell aplasia. You know, the heroic work that was done from the companies involved with EPO and the neutralizing antibodies there. There is an alternative approach to kind of take this to kind of understand the area collectively. And that's by establishing a reference antibody preparation. And, or reference samples, patient samples. This way you can use that type of approach to understand how the assays are performing relative to each other.

And in terms of collaboration, that's something

that we've been working on for the last while. In collaboration, Genzyme with Shire and the NIBSC to establish a reference antibody preparation in Fabre disease. That there is, so we're making progress in that area. But that is a approach that could be taken. And that means getting enough patient samples, it means getting appropriate informed consent and all, but that is a approach that could be considered and discussed further.

MALE SPEAKER: Sue, before you go, can I ask just one, question of clarification? Those kinds of standardizations, is that all referring to just like measuring IGG when in fact what we're more interested in are these other things that are assayed differently? Like percent inhibition or percent uptake? Those are much more complex assays, but none of the ones that you're referring to if I understand correctly. Maybe I'm wrong.

MS. RICHARDS: So, the inhibitory antibody for uptake or enzyme activity those are subpopulations of antibodies for binding antibodies. The binding antibodies is like total. And the other two are sub-populations. So those are assessed in different assays. And as long as the sub population of antibodies is available in the reference preparation you can totally use this approach to do that also. So you would end up with different titer numbers.

Probably much higher for binding antibody titer in this reference preparation and a lower for the inhibitory or the NAB assay. But the approach is still feasible.

MALE SPEAKER: I'm Young Chang Cho [spelled phonetically] and I'm representing Shire. So I agree with what Sue just said comparing different platforms or different assay formats. It's very difficult. But, having said that, you know, it's very important to make sure that a continuity is actually there. So in terms of monitoring these antibodies for patients, actually at Shire we also provide these commercial testings to the communities and in fact trying to, for us, trying to make this actually a faster response and more sensative assays. Some of these older products, for example, Airaprise. We have very complex testing schemes in the very beginning. And now we have learned a lot more. We're actually trying to make more sensitive and faster assays and then, also CRO's to provide testings through the communities so that the turnaround time would be much faster through the patient community and the physicians who request those tests.

In the meantime, because we're changing these assay formats. So internally we're doing a lot of research now trying to make sure that the continuity is actually there so that people don't get confused by the pure titer

numbers because the assays now is more sensitive than before. And so it doesn't mean that it's really much more severe than before, but it is important to really look at the correlations between them. So, in the very near future we will actually provide more information to the whoever requesting testing will receive letters from us explaining these differences as well. So the overall experience is that we're just trying to provide a faster and more complete service to the community. You know for that regard.

DR. KISHNANI: The next.

FEMALE SPEAKER: Hi, my name is Tressa Allington [spelled phonetically] and I'm here from Alexion. I'm a scientist who works on these assays almost exclusively. And I realize that this is not a assay centric conference and so I'll try not to go into too much depth, but all of this talk about the titer is very interesting to me and I just wanted to add something. There's a slide put over earlier about the immunogenicity assay paradigm where you do the screening, confirmatory and then characterization and then distillation steps. The titer assay comes after the confirmatory assay typically and it's done differently by different sponsors as to how the samples behave. It's based on a titer cup point, which is when you serially dilute the sample the titer's reported based on when you crossed that

cup point. And for some assays, that's the same as the screening cup point.

And for other patient populations, no matter how much you dilute that sample you achieve an aceomtotic signal and you never cross that screening cup point. In those cases it's often a multiple. Like one and a half times the screening cup point. So unfortunately, it's all done a little bit empirically. And, very specific to the molecule and to the patient population. And I think it would be a great thing to achieve a more standardized approach to that. And I've done some literature reading on that myself. But I think that from the physician's perspective, what might be really helpful is conveying some of the historical performance of that assay across clinical trials or in the post marketing space to give the physician that information as to where is your patient falling with the range of Pompe disease for example. To give some context.

DR. KISHNANI: Yeah, I would agree with you on that because often what you get is a sheet and a number. And there's no interpretation and so you're left with guessing, is this, you know, for the majority in this room there's experience with these [unintelligible] diseases but many of them who are getting them infused out, this has no value. Or it's meaningless. So adding that component of

some relevance or lack of relevance or needs follow-up would really be very helpful, at least for me as a clinician from that standpoint.

DR. BLUESTONE: Priya, can I make a comment on that?

DR. KISHNANI: Absolutely.

DR. BLUESTONE: I think this just emphasizes the need for doing some research in this area and I think this is a great opportunity for a group of people in NORD or somebody else to organize a workshop to be able to do this. Let me, let me just sort of give a little science around what you just said. We, these assays are all testing a polyclonal response. So if you have one antibody that's present in a titer of one to 50, that's in there against a certain epitome, its' going to dilute out very quickly. And you could have another antibody that's got a titer of one to 1,000,000. But that antibody is only 10% of the antibodies so you'll get to your cut off point very quickly. Because the one to 50 dilutes out and the one to 1,000,000 antibody is just going to keep going on forever.

Well, which antibody was the more important antibody when looking at efficacy or the impact of it? We don't know. Right? So, I think until we do a lot more science to try to understand what the nature of the

antibodies are and what they do; a simple titration which is all your doing of a polyclonal response is not going to necessarily correlate. Which is what we heard earlier today, or be, or be functionally useful information. So I think this, your point you raise is really excellent. And just demands that we do more science around this.

FEMALE SPEAKER: That's certainly right. The number that you get is an amalgamation, let's say of all of those antibodies contributing to the titer. So, thank you.

MALE SPEAKER: Gary Totingish [spelled phonetically] with [unintelligible]. Just a comment to Dr. Bluestone's suggestion of research. The challenge there is either, you know, you either have that in a University, which it isn't that interesting in some cases someway, or you have it in industry. And industry won't usually do it unless it's a big safety issue or there's a return on investment kind of thing. So, those are challenges to fund that. I appreciate it. I think it's a great idea and I hope someone can do it.

I'm here to say just Biomet takes the same stance as Shire and Genzyme in how we do our assays. I think one thing that needs to be clear. I think someone showed some percentage of antibody responses on there and some assays are total antibodies where they are what's called a bridging

assay. So it measures all kinds -- IGG, IGM, and so forth. Others are just IGG non sub-type specific assays. So they're only measuring IGG. So how you interpret that might be different than how you interpret a total antibody assay. Just clarity on that. I think, I've been doing this for many years with these ERTs or with antibody therapeutics and other proteins therapeutics and I think it's really important that we make it clear that these should be per molecule understanding. Not across molecules. And hopefully that is getting across in this discussion too.

So, one other thing I think, I just wanted to make -- another challenge is that we talk about CRIM positivity and CRIM negativity but there's also the issue of preexisting antibodies. Some of these patients actually have preexisting antibodies that we see in our assays depending on how you look at it again your cut point. Right. You see some preexisting antibodies. How does that impact the patient and antibody formation too? So I think that's another thing that is important to understand throughout this.

DR. KISHNANI: I just had a question still for many other industry representatives. Whether now, as drug development is proceeding, how you are incorporating this into your system. And Charlie, I'm looking right at you

from Oxrain if you would like to comment and then others as well.

MALE SPEAKER: Well, I'll just take a deep breath and say I think firstly avoid your question a little bit and say, between the different diseases I agree with the panel. So being able to look at different antibody titers in MPS two or MPS one and trying to interpret those in light of a titer number in Pompe's going to be impossible. But I do think in industry the efforts from Shire and Genzyme within a disease to look at antibody levels, uptake assays, activity assays is really important because you can imagine, physicians it's a natural thing as they are presented nowadays with more than one enzyme replacement product within a disease they're asking question like is one product more efficacious than another. And a natural extension of that, is one product more immunogenic and if so, is that product affect clinical efficacy.

And I think efforts to help standardize this and bring the companies together to do this properly so that you can turn around and give that information back to physicians ought to be applauded. You can imagine this is touchy information for companies and so this idea, people ask for "could you just immediately put this information into a public database" and I think you know that the marketing and

sales people have a cataleptic attack about just putting this information out there without, properly so, without the same kind of interpretation, the same kind of scientific studies that go into look at that.

So, I think with all rare diseases as we move from isolated anecdotal cases and from clinical trials in the real world settings I really encourage those kinds of efforts to be done within a disease. Within an enzyme replacement, even second generation molecules. Thanks.

FEMALE SPEAKER: So Priya --

[talking simultaneously]

DR. ABICHANDANI: One point I'd like to make is that industry has come a long way compared to where we were a few years ago. As I showed on the slide, I think certainly at Shire, and Genzyme, and Biomarin, they are certainly moving to that tiered approach. I think it's very similar. All of the companies are doing it, approaching immunocity in a similar way. And in terms of how are we doing it and when are we doing it, I think we are looking at assays early in development and certainly planning to have validated assays in time for pivotal development. I think historically there's been a lot of variability but I think going forward I think we recognize there needs to be standardization. It needs to be more rigorous. So I think

that we've certainly made a lot of progress in that regard.

DR. KISHNANI: Anne, I think you had a question.

DR. ABICHANDANI: Anna and Sue can correct me if I'm wrong, but I think that's the approach that Genzyme and Biomarin are also taking.

MALE SPEAKER: I just want to ask another question. Since we are talking about isotypes and so on, I think Susan mentioned looking at isotypes. What benefit do you see, maybe Dr. Bluestone you could comment on that, knowing the isotype?

DR. BLUESTONE: You could imagine a few things. I mean, if you are thinking of antigen uptake as a major mechanism by which to affect the efficacy of the drug, knowing the isotype of the antibodies you are making can be key. Not just in determining how much of the complex will be taken up, but in what type of cells it might be taken up into. So I think there's clearly an important need for isotype. The other thing is it will tell you something about the maturation of the immune response that might be occurring over time. And as you learn about the maturation you might be able to identify, assuming that there's some point in which one's going to think about immunotherapy, which one's going to be the best immunotherapies.

Because, for instance, we'll hear some data on

Retuxan or proteasome inhibitors and knowing whether you are looking at a B cell, long lived B cell response, or you're looking at a plasma cell response, the isotypes will tell you a lot about that as well. And then finally, I'd point to that the isotypes may tell you something about the nature of the type of T cell response that you're getting. Which again, I know I said this earlier, but I think we're looking touch of the fact that there's more going on here than antibodies being made. And knowing about isotypes might tell you about the type of T cells that are responding. So called TH one or TH two which may also impact on the degree of inflammation overall that's occurring in these patients that are being treated with the protein.

DR. KISHNANI: Anne, I think you had a question or a comment?

DR. PARISER: Yeah, I'm actually going to segway into a slightly different topic if that's okay. So, just returning to the more clinical realm, obviously there's a lot we don't know and there's a lot more information that we need. But, I think what we do know is that he CRIM negatives are a particularly severe genotype and we should expect that they're probably going to have high titers and possibly neutralizing effects as we've certainly seen with Pompe disease. So, a question then for the group is, are

these people that we need to be studying early with drug or drug plus immune tolerizing regimens really right out of the gate. I mean this would be very very useful information to have and you probably wouldn't need that many patients. So, I guess I'd just like to throw that out to both industry and the clinicians if you could please comment.

DR. BLUESTONE: I'm neither industry nor clinician, but if it's okay to comment anyway. I would think that would be exactly what you would want to do. I mean, there are, I know this is more a topic for the next session and I'll talk about this myself, but this is much harder to deal with immune responses that are established and to prevent, then to prevent an immune response from the get go. So I think it's an easier hurdle to try to get over immunologically. Doing that may help you then with more difficult patients later on. It's just sort of a common approach to trying to get over the lowest barrier first and then use what you learn there to get over the next barrier. So I think it would both be easier to do for that group of patients and then potentially informative for the next. It may not take that many patients as you point out. Although there's always the risk, as we talked about, if you know, what you do in a few CRIM negative patients in one disease may not translate into what works in CRIM negative patients

in another. But it's a start. And I would think that would be the right place to go.

DR. KISHNANI: Amy, I think you have a comment.

DR. ROSENBERG: So, but doing that right from the get go I think that would also allow you to see whether or not your enzyme replacement therapy, what effects it has. Because you've removed the immune response as a potential blocker to it and so then the question focuses more on how one can maximize the efficacy of the enzyme replacement therapy. And I think that's a big jump start, rather than having to go back, [unintelligible] patients and then you know, hope that you can see something after that. So, I agree fully.

DR. MUENZER: I think one of the big challenges is that if you go beyond Pompe, where I think we see the very obvious negative effects of lack of efficacy, in the otherwise soma storages, there's not that same timeline of severity. It's much more difficult to assess, you know, with bio markers with urine gags for example, for the MPS's, what's really going on. There's joint range of motion, cardiac disease, they're much more subtle and much longer term in terms of changing.

And so that's one of the challenges we have, I think, in terms of if you immune suppress people how do you

know, you put them through that inherent risk when you may not need it and it may take a very long time to show that there's a difference. And without doing some sort of control, it's really, I think, challenging. So, I agree, I think the immune tolerance is an issue. But the downstream, how do you determine efficacy of what we do is really challenging. For a lot of these patients for example, the MPS patients who are cognitively impaired, they've never been studied in a clinical trial because there is no end marker one can use that are validated. And so that, I think we need more other biomarkers. But I think that's the challenge, I mean. But I agree with you. It would be nice to do that. But how do you know what you're doing once you've done it?

DR. ROSENBERG: So, I would agree, the clinic end points for many of these diseases and outcomes of efficacy are difficult to define. Yet, from again it's a preponderance of data and those animal models are especially helpful. Why would you not want to optimize. Optimize the therapy. Regardless of whether you know, you know, you have some outcomes which are truly meaningful. Don't you want the best possible outcome for these patients. And if so, if the tolerizing protocol is safe as you will see Priya will present, you know, it would seem to me that given all of the

evidence, that's what you want to do for your patients. Give them the best chance.

DR. MUENZER: Absolutely agree, we want to give them the best change. The question is who to select to give them the best chance. Are we just taking CRIM negative as the start?

DR. KISHNANI: Right.

DR. MUENZER: That's one of those challenges.

DR. KISHNANI: So Joe, there's some comments that I would love to make. And I think yes, looking at the so called, the CRIM negatives and these other populations, if you look as you said for MPS one and MPS two, the clinic trials were done more in the, across the spectrum where you know there was some residual enzyme activity. But yet when you look at the paper from Dr. Raith [spelled phonetically], you look at the example from MPS two from Dr. Burton, these were both, these were situations of what we would call CRIM negative. And so, I think starting to establish even understanding the genotypes and how that translates into is there any protein expression; starting with that subcategory of patients as a starting place for the risk benefit. I think we are at a point in our field where we need to start looking at that next step.

FEMALE SPEAKER: The other issue with starting an

immune tolerance program early in the clinical development of a potential enzyme replacement therapy is that you're introducing confounders for safety and you really need to be able to tease out the different, the potential adverse events from these drugs which are not at all safe from your ERT. And the other question is what other types of things might you mask from your ERT during your clinical drug development program that you might not see because you've immune suppressed this child.

DR. ROSENBERG: Priya, can you restate the question, I didn't quite get --

DR. KISHNANI: I think the question that Patty has is that if you have other drugs in this like immune modulation then you're confounding what's going on with ERT and some of the potential side effects could be related to these ITI regimens. And also the downstream effects from it. So, the question is how would you design a clinical trial and separate these two components out.

DR. BLUESTONE: Priya, I could try to address it a little bit. It just seems to me that we're talking about a step wise process here, right? I'm not sure that you would necessarily want to be thinking about incorporating a telegenic therapy during drug development which is what you were referring to. But I think looking at drugs that have

already been approved that have some sign of immunogenicity. So I agree with you, you don't want to complicate drug development in the early phases. But I certainly k that once a drug is approved you are provided the opportunity to do this.

I'd make a couple of other quick points. I think there's a really interesting part of the conversation and Larry hit it on the head. Typically in these kinds of settings we add on therapies that we think will be beneficial and we're pressured often to do it in the sickest patients first. Right? And in the particular case that would be a backward way to develop this. Because those individuals, first of all have very different immune responses, they have high titers and they may be more difficult to tolerize. So I think we need to think differently about that as Larry pointed out.

I think the second thing which is I think this CRIM positive CRIM negative thing, it's driving me crazy so I guess I'm going to have to say something about it. I have no idea what those words mean actually. I'm not sure what's cross reactive on what. If a protein is truncated and is missing a piece of it, you could be making a de novo immune response against a missing piece which is no different than if the protein is missing totally. So that's not cross

reactive, that novel. I think that, getting back to the earlier comments, until we know a little bit more about what's the immunogenic part of the protein in these people it's hard to know which are the most appropriate population.

I would argue that from a tolerance point of view, you want to be treating patients who are making a de novo response, against whatever it is. Whether it's the missing part of the protein that's been truncated or somehow altered or a structural allestaric effect of having a mutated protein that has a different confirmation. But I'm not sure I would be so, I'm not confident that I would be so capable of saying "well this patient population is the best one to use because it's either CRIM positive or CRIM negative." So I think it requires some thought but I think there's some real opportunities to look early on and I would encourage people to think about how to really test this question. And my last point is, I understand the challenge of not having a great clinical end point.

I think that's just such a really important problem. I think what I would say though is that if I told you the drug was totally safe and free no manufacturer, no drug company would want to be giving an enzyme that they thought might induce an antibody response. So under the

right circumstances of confidence and safety and low cost I think that anybody would want, who could avoid an immune response against these proteins would want to do so.

DR. KISHNANI: Thank you Jeff. I think that's really helpful. Because yes, the word CRIM drives me crazy because it's a spectrum and it's beyond sometimes just the genotype, it's a number of host factors and other factors also that play a role in the immune response. But if you start at the level of the most severe or sick or involved you may start teasing this apart.

FEMALE SPEAKER: And if I could just say, I think the important thing here is not necessarily whether we say we have to start in CRIM negative patients or CRIM positive patients. But really that it's an inclusive whereas in the past a lot of it has been CRIM positive. So, whether it's a sub-study or parallel study when you look at both I think that's really the important aspect. DR. KISHNANI: I think Anne has a comment and then Joe, you can go next.

DR. PARISER: Yeah, I mean I think that for the severe genotypes, or people that we suspect certainly are going to do badly, I think studying this in the premarket period is really worth discussing. And we can revisit this in the immune tolerizing. And yes, you have some confounders in there. But I think with small patient

populations what we really want are facts. We want facts that we can put out there and then continue to gather as we go along. But, I don't think that we should exclude that. I'll just put somebody on the spot. Is Julie Bites [spelled phonetically] still here? I think she had wanted to comment on this a little bit.

FEMALE SPEAKER: So, yeah, I guess I was just going to say much of what Anne just said. Which is that we are open --

FEMALE SPEAKER: Julie, we can't hear you very well.

FEMALE SPEAKER: Okay. We would be open to information about the efficacy of combination, of the combination of an ERT vs. ERT plus immune modulation in patients who you would anticipate from the get go are going to be bad actors. I don't think that we would necessarily require that post approval. I think that is something that you might want to do as early as you could, incorporating it into your development plan. Perhaps as an additional arm in a trial you are already doing. Or as a sub-study of a study that you are already doing. I think there are creative ways you could incorporate or attempt to answer the question in a few patients because if it's going to work, you're not going to need a lot of patients to show it.

DR. KISHNANI: Joe.

DR. MUENZER: Can I just actually follow up with Dr. Bluestone and ask him the question how practical it is to do these epitope typing and what sort of, is that relatively easy to do these days in terms of figuring out the nature of your polyclonal antibodies are and how many different epitopes they're recognizing? From a practical point of view. I mean I know in theory that it can be done but how readily available is this sort of technology and the resources to do that. Because right now that is one of the challenges in this rare disorder. There's not a lot of interest in doing that very practical aspect.

DR. BLUESTONE: Yeah, I don't want to suggest that it's trivial. But I think with new molecular techniques it is relatively straightforward to, and especially if the community would do this in a coherent, cohesive away, relatively easy to make an array of mutations that can be monitored or truncated proteins that might reflect what's happening. These would take a couple of months you know, maximum, to make these proteins and to set up the individual assays to look at it. Including epitope binding and the like.

So I don't, it's not, there more -- it's less about money and more about will. If the will is there it's

really not that difficult to do that. And then, and this gets back to lots of the discussions earlier in the day and even the most recent one about how do you test a new drug in development. I mean if it was type one diabetes it would be a no brainer because I have a million patients I could be looking at. In the absence of a large degree of, number of patients, it really will require a collaborative effort.

Even to do a multi-arm trial and have enough power to show a difference in efficacy with and without toleragentic drugs, it sounds great, but having been involved in this a little bit, as have many people in this room, is that you can be mislead by having anecdotal data. So if you really want to do a good trial you have to have a lot of patients and that's going to be a challenge.

DR. MUENZER: And that's really one of the challenges for these rare disorders and certainly some of the disorders that are being considered in the future there are even less patients and it's even more difficult. I think that's one of the, we have to be aware that any precedent set is really going to impact even more and more the development of even rare enzyme replacement or therapies for even the rarer disorders.

DR. KISHNANI: Dave.

MR. SCOTT: David Scott, Uniformed Services

University. I just wanted to get back to the comment that Jeff made about CRIM and the definition and how it drives some people crazy and how it's actually misused. It certainly shouldn't be used in terms of making predictions. I do a lot of work in hemophilia and the patients who are problematic there are those that make antibodies that inhibit the activity of the clotting factors. And of course, when I went to then the first, the first hemophilia meeting that I went to I had no idea what the word inhibitor meant. Then I realized that it meant an antibody. And it's just a functional definition.

And I think we have to be careful even about using those. Because we can have patients in hemophilia, the most common mutation that leads to "inhibitors" are major deletions or inversions where there's very little of that molecule that would be "CRIM" that the patient has. But you could have a single point mutation that leads to a dysfunctional molecule. And they are clearly very CRIM positive by that definition and you can make an inhibitory antibody. So I think we have to be very careful and not get into the semantic problem and making predictions in terms of who to choose for tolerance induction.

DR. KISHNANI: Thanks Dave. If I could just make a comment here. I completely agree with you. It think if

we are taking the tip of the iceberg then at least we know whatever we are calling as CRIM negative, the one's with the deleterious, we know that the vast majority if not all are making it. So when you're doing trial design and you're trying to check or test your hypothesis then that might be a sub-group of patients to look at, knowing full well that you may be missing some patients with a [unintelligible] mutation or the point mutations and that they could also be making high antibody titers. I think that was one of the comments that I understood from you.

MALE SPEAKER: This is Charlie from Oxyrain. I just wanted to agree with Jeffrey. I think when you are considering putting together clinical trials for these. Especially with the ultra orphan diseases, which many of these new therapies are for enzyme replacement therapy, imagining that you can have separate arms within without makes it very difficult in imaging enrolling patients. Now having said that, you know, my inclination is you know for the hurler patients, for MPS two patients from many of the ERTs where there's no cognitive involvement and very little enzyme, I agree with Amy that really what you want to do because none of the ERTs are curative, you want to maximize the clinical effect. You'd like to be able to show that early and not have to you know, use the first four or five

years of clinical trial showing that again, patients that have high titer antibodies have neutralization of clinical activity.

So I have a question for Priya, I think in Pompe disease we have really good evidence in CRIM negative patients, whatever that means - that's a dirty work I guess - but that immunotolerence regimens are highly, can be very effective. But you've also shown in the CRIM positive patients that there are patients with sustained high titer antibodies which can be very devastating to the patients. And knowing what to do as a drug developer and thinking about that, not CRIM negative but CRIM positive in Pompe or some of these others where it's hard to predict ahead of time whether we will have mutations or deletions. It's kind of this zone in the middle with what you want to do with that. The patients that do develop the antibodies have really bad outcomes, but maybe you wouldn't have had to do that. We can talk about this afternoon, but I'd like to hear more about the evolution of your thinking as you find more and more patients with partial enzymes that really do have high titer antibodies which really do negate the effectiveness of treatment.

DR. KISHNANI: You know, I can try and address that in the afternoon. But clearly I think the idea is if

you can develop a regimen that's safe. Then the idea would be to try and develop something to go along side enzyme replacement and not play this game of waiting. What is the mutation, should I be treating with an immune tolerance induction regimen or not. So I think that's where our thought process should be. Can we develop something that safe, it's short, and effective across different diseases. Really my work is just a very small piece of the bigger picture of this field. But I think that is where my thinking is especially as new [unintelligible] is coming on the horizon. We're going to be picking up more and more. And if we want to treat early we've got to learn a number of lessons and try to develop safe therapies.

DR. BLUESTONE: Now if you do that in a uniform way, we talked about that in clinical drug development you don't wait 'till post marketing and that. I'd be interested to hear from Anne or Amy about how that affects the label. We sort of said that and we moved away from that. But it seems to me that if that's the patient that you are treating and you have a, what you feel is a safe and short effective course of immunotolerance then that necessarily has to be part of the label. And that is something that in tertiary care medical centers where people with a lot of experience with this feel very comfortable about that.

I think when you get out into the real world of centers that maybe don't do this all of the time, how you imagine this playing out in the real world with the label. Instructions to physicians.

DR. PARISER: Well I suppose I can give it a try but I mean there's a couple of places that you could put it in the label and it would depend on the strength of the data and what you have. Probably the clinical studies section where you're describing what was done and what the results are would be very suitable for putting those in.

But, I think what we've also heard from some of the clinicians earlier is that often they are incorporating practice of medicine and trying different strategies that haven't' been tried, as you know in tertiary care centers where there's a lot of experience with this. So there's only a certain amount of this that's going to be able to be labeled. But if its' facts and we have data that does at least present the opportunity for including it somewhere.

DR. BLUESTONE: I think the problem is that if you go ahead and take the leap of faith and you go ahead and you incorporate imunotolerance protocols in much of what you're doing for these rare diseases then you may not actually, what if a physician says "Well I don't want to do that, that's not what the evidence is, that's not what's in the

dossier." You know, not having a separate arm, where you haven't done immune tolerance, it's going to be hard.

DR. PARISER: Yeah. I guess that goes back to the plea for more information.

FEMALE SPEAKER: So I had one question, and perhaps we covered this in the afternoon, but does this get viewed as a combination product? You know, combining the enzyme replacement therapy with an immune tolerance regimen. And I guess the other thing is, these regimens are not approved for this. I mean I understand that it's a challenging situation, but they're not approved, they're not studied the different immune regimens. How do we standardize this across all ERTs? I think that's the question from an industry perspective that becomes a bit challenging to understand. And, again, just to echo, these are small populations so again, for a smaller arm, it won't be powered, obviously, to show -- if it's a very dramatic impact then I think it's' easier to see. But if it's more subtle I think it might be harder and I think, I'm not quite sure how we do that or develop the drug in that scenario.

DR. PARISER: Clearly these are a challenge and I don't think we are going to be able to answer that today. So --

DR. KISHNANI: So we've got just the last 10

minutes and so, [unintelligible] I mean if there are any other questions or any other topics that we would like to cover it would be wonderful.

MALE SPEAKER: I guess I was just trying for myself summarize you know the conclusions of the various comments and I'm very concerned about the ability to test tolerizing protocols post marketing. Simply, there's not the money for it. There is no probably NIH grant that is able to put together enough money to do that in a proper manner. And likewise, if we ask that as a registration criteria, to analyze a tolerizing protocol I would be concerned that some of the other panelists have mentioned that it could be conceived or written into the label as a concurrent therapy or an indicated therapy and that makes it actually very, very hard to apply to insurance companies when you are using that product.

So I think, I would plead that there be strong consideration for these potential aspects of therapy and that there is some consideration of that as the drug is going through FDA registration. But perhaps the best way of getting at the point when these very, very rare diseases, ultra orphan diseases, are being studies is to report individual cases as we're doing here, have meetings like these where we discuss the topics and can learn about the

basic science but getting the data in front of the industry that might be out there also would be tremendously important.

DR. KISHNANI: Chad I would really agree with you because even in our work with Pompe it's very hard to get NIH support and it's really been sheer will power trying to do this and so it would be wonderful if industry recognizes this and also provides some infrastructure support for clinicians and academicians who are interested in pursuing this further I think would really help move this field forward. So, I actually see Dr. Majestki [spelled phonetically] here and knowing that he has been part of industry and now is part of FDA I am going to put him on the spot and ask for his words of wisdom, and he's also an immunologist, of how he sees thi and what's the best approach.

MALE SPEAKER: I knew I should have snuck in very quietly. So, when we first began to develop a therapy for Pompe's at Genzyme before I came to FDA, and we initially saw a very high rate of antibody response, we thought that perhaps we should in our development program have an arm that attempted to tolerize. I think at the time, of course, it was difficult to know what that tolerizing regimen should be. We've obviously come a very, very long distance since

then and learned a lot. But you know, as challenging as the issues are, when initial phase one, two studies begin to uncover very high rates of antibody response such as that situation and if we do see these signals that can in fact lead us to consider that those patients are at high risk then I think we must consider this kind of development program that would incorporate a possible tolerizing program.

Having said all that, it's, we can say that today only because we've learned so much about these tolerizing programs. I think we have a lot to go before then. I do think that industry has an interest in these tolerizing programs and can be supportive. The cost of the tolerizing regimens though, are substantial. But I think many of the companies would probably be willing to consider funding at least a significant portion of these kinds of studies. Now, I don't know if that answers your question, Priya, but I'm happy to opine on anything else. DR. KISHNANI: No, what I'm coming to is that when we started it happened that the pivotal trials for Pompe was in infants. In many of the other diseases the pivotal trial is not in the infants. And so we've already missed that boat because we've already made some claims that "my enzyme does not make antibodies." And then you're starting to treat that next set of patients that

did not meet your clinical trial criteria and there is no collective experience on it. Or, then we have the challenges that we say are of end points. But, what I would like from your perspective is how important do you think, and I think we're coming to that conclusion, but it would be wonderful to hear it from someone like yourself because you've seen it from both sides of the coin.

MALE SPEAKER: You mean in terms of the importance of immune tolerizing regimens at the beginning of therapy for patients?

DR. KISHNANI: Right.

MALE SPEAKER: I think absolutely. I think it's critical today some of these, Pompe being perhaps the poster child. But it's not the only one where I think this would be extremely useful. I really don't think we know the extent to which treatments in the MPS group of diseases can really, what kind of response they can really achieve today, without perhaps, considering early immune tolerizing procedures. So, I'm a big proponent of moving forward with this.

DR. KISHNANI: Thank you. Anne or any other the panel members, any last comments or questions? We've got a last two or three minutes.

[inaudible commentary]

DR. KISHNANI: Absolutely.

DR. TANPAIBOON: I'll make another clin-pharm kind of pitch here. As I heard lots of discussions about the herterodginy of the polyclonal reaction to the protein therapies, ERTs, it becomes clear that you have to develop and assay to account for all different types of antibodies. And that's labor intensive and at the end you've got to stitch it up together with other data. So I'm wondering if there's a secondary tool that our clin-pharm colleagues that are in the analystica laboratories can try to develop. Thinking in terms of the inhibitory antibodies, if we have a drug with the uptake domain, we have a drug with the activity domain and the inhibitory antibody is binding to one of those domains. Can we develop an assay which were going to tell you, if I look at the drug concentration I know that the inhibitory antibody exists. Right? So that may be an earlier tool that we can develop in the analytical laboratories to kind of give a little bit more heads up in the clinical development stage. Just a last thought.

DR. KISHNANI: Thank you. Anne, should we wrap this up for lunch. So I think there's lunch from 11:30 to 12:30 so we're going to reconvene back here. Thank you so much.

[off the record]

Session 2: Role of immune tolerance induction in enzyme replacement therapy

DR. DICKSON: If everyone will please take their seats, we're going to get started with the second session. Okay, welcome to Session Two of the workshop on "Immune Responses to Enzyme Replacement Therapies: Role of Immune Tolerance Induction." I'm Patty Dickson at Harbor-UCLA. This is Anne Pariser at FDA CDER, and we are starting Session Two "Role of Immune Tolerance Induction in Enzyme Replacement Therapy." A couple of quick announcements. First of all, this discussion is going to feature off label discussions, and also, we are, I am told, on Twitter. I guess we don't have a unique hashtag, but people -- oh we do?

FEMALE SPEAKER: I-T-I-E-R-T is what some have been using.

DR. DICKSON: Okay, are we trending?

[laughter]

FEMALE SPEAKER: I don't know that we're trending yet, but I'm trying.

DR. DICKSON: Okay. And apparently some people have been using #raredisease to talk about us. Okay, any other announcements?

FEMALE SPEAKER: No.

DR. DICKSON: Okay, think you're the first speaker.

Pharmacologic approaches to immune tolerance

DR. PARISER: Okay, well, good afternoon and welcome back everyone. So, I'd like to introduce our first speaker. Well, actually we have another very exciting session for you this afternoon, and we will be featuring, actually, five speakers. We've had another addition. Melissa Hogan will be offering an additional patient perspective, and the testimony she'll be reading will actually be available later on the website, so it will also be posted with the other materials. So that's just one last minute agenda. So, then I'd like to get started and introduce our first speaker. This is Dr. Laurence Turka. He's the Harold and Ellen Danser Professor of Surgery at Harvard Medical School and co-director of the Transplantation Biology Research Center at Mass General. He's an internationally recognized expert in the areas of immune tolerance and transplant immunology, and he has been involved in the immune tolerance network since its inception, and is currently the deputy director of biomarker and discovery research. So he will be talking on the pharmacologic approach for immune tolerance. So thank you Dr. Turka.

DR. TURKA: Thanks very much, and I appreciate the opportunity to attend and to learn more about this field,

and hopefully to teach a little bit as well. So, I -- let me start with my --well, there's my disclosures. Maybe most prominently, my wife is an employee of Novartis. So, I was asked to talk about pharmacologic approaches, and to talk a little bit about what I would think of as a definition of immune tolerance, and I come to this from the perspective of somebody who's been working in transplantation and auto-immunity for a long time, and to give some examples of where immune tolerance has been successfully induced in these areas. And so I'll try and do that in a relatively brief period of time.

What do I think of as immune tolerance, and I think Amy Rosenberg put this up earlier. I would say that it's the absence of a deleterious immune response, and I have put deleterious in italics, because -- for reasons that I'll talk about later. I think that tolerance as we think of it, certainly in the fields in which I work, is accompanied by an active regulatory immune response that prevents pathogenic or pathogenicity, so I think it's the absence of a deleterious immune response without a need for ongoing therapeutic intervention. And so, there, as I say below, again, I think that tolerance can be, and usually is an active process with accompanying protective immunoregulatory responses.

So it's -- I think it's always interesting to think that when we look at these responses -- and I'm going to take a little bit of a T cell-centric view, because, as Jeff Bluestone alluded to earlier, although we're really looking primarily at antibody responses as a read out, in most cases, and I suspect the same is true here, the antibody responses initiated by a T cell response that provides B cells with the signals they need to make antibodies. It's instructive to realize that all these responses come from a very, very small number of cells. They're probably in the order of only a couple of hundred cells, T cells, in humans that are able to respond to any given epitope of a foreign protein or a foreign peptide, but they respond very, very quickly. And you can see here these small numbers of cell -- and maybe the pointer doesn't work. Oh there we go. They'll have a doubling time of about six hours, and within literally a week they can multiply tremendously. And this is a schematic from the course of an infection. And then as the infection is cleared these cells will gradually disappear.

And maybe I'll see if I can make this slide disappear. Anyway, the way I would think of it coming from the tolerance and auto-immunity field is that establishing tolerance to any antigen is a balance between the ability to

delete those cells, because you can generate many, many antigen reactive cells, and the ability to generate a regulatory cell response. And then the absence of deleting many of these cells, the normal counter-regulatory mechanisms of your immune response will be unable to counteract these pathogenic cells, and you'll get an autoimmune response, or in the case of the area in which I work a lot, organ transplantation, you'll get rejection. If these cells can be curtailed and culled -- not down to nothing, but down to some manageable number -- then regulatory processes have an opportunity to work and tolerance can be established.

When I was thinking -- I asked to think about what approaches might be, and so one of the things that I was asked to talk about is what's available out there, what's been used, and what are the kinds of things that people should think about. I've tried to divide them into three broader categories. Let's say that approaches that are antigen-specific -- so you can think of delivering antigen in some form that's inherently tolerogenic, or relatively tolerogenic. One is soluble protein or peptide, and it's actually one of the things that I think we might discuss later, is that we think of very young infants as being relatively immune compromised; we don't typically vaccinate

them very effectively until they're older, and we're giving them enzyme replacement therapy, which is protein or peptide in a very non-immunogenic form, which is intravenously. So why do they generate immune responses, or do, in fact, all of them generate immune responses?

So that's one way that I would think about it. Peptide can be coupled, potentially, to MHC molecules, which under varying circumstances may be toleregenic. It could also be coupled -- and I say peptide, it could be protein here as well -- to a drug, and they can possibly be linked, such as in a nanoparticle. So, these are all examples; they're not meant to exhaustive. You could think of others that would be antigen-specific. You could also think of antigen nonspecific therapies, which are designed to block signals that are necessary to initiate or to sustain an immune response, and assume that the antigen delivery is occurring endogenously, in the case of self-antigens, or exogenously is being supplied in another format in -- as ERT.

And then here, things that might be blocked, and have been tried in various stages or being studied, are blocking T cell co-stimulatory signals, which are also important, we know, for antibody production, or blocking inflammatory cytokines. And then, another approach -- and

these are not mutually exclusive -- might be to deliberately deliver negative or regulatory signals, such as cellular therapy with a regulatory T or potentially regulatory B cells. Low dose interleukin 2, which can expand regulatory T cells, or await the development of reagents that can activate negative regulatory pathways. So we know a lot about blocking these pathways for tumor immunotherapy, but I think a number of people are thinking about, can these pathways, such as PD-1, be activated to inhibit immune responses.

Now, I was asked to talk about real life examples of tolerance in fields in which I work, and there's not a lot, and I'll tell you about an attempt that didn't work and an attempt that did work, both in the same field, because I think that they're instructive. So, when I started working in this area, which is a long time ago now, there was this molecule CD28 on T cells that was identified as a key molecule to provide co-stimulatory signals to enable responses to go through. And Dr. Bluestone was working on this as well, and in fact, before we became friends we were engaged in a, I think, friendly competition, which I think lost and for priority. And a reagent was developed called CTLA4Ig -- which is now Orencia -- that showed that you could block this co-stimulatory signal. And Jeff's lab and

our lab and other labs showed that you can induce transplant tolerance in small animals, and that was about 22 years ago.

What happened was, though, when this approach got translated into non-human primates and into people, it was shown that, in fact, this didn't work very well, because while blocking co-stimulation deletes antigen reactive cells in mice, it doesn't -- one effect that it doesn't have is induce tolerance in non-human primates. So, as I said, it's FDA-approved for rheumatoid arthritis, and a variant is approved for renal transplantation. So, what was the lesson that was learned? That it didn't work in small animals -- that it worked in small animals, but it didn't work very well in people. Well, humans are not just big mice. We probably knew that to begin with. And non-human primates and humans are exposed to many pathogens, and they have many memory T cells, which we could think of in a sense as an equivalent almost of plasma cells for memory responses. We know that these cross react to transplantation antigens, and memory cells are hard to delete or hard to suppress, and that's a problem for pre-established responses to auto-antigens as well in the case of autoimmunity, and it's obviously going to be a problem, therefore, for enzyme replacement therapy when there's already been a T cell or

cellular and antibody response generated.

This is just an example of an experiment with this where we tried to model this in mice, and we can show that, if we took animals that got the blockade of this signal and we didn't put any cells into them, we could get long term transplant survival, and if we infuse them with naïve T cells we got long term transplant survival. But if we infused them with a very small number of memory T cells, long-term engraftment couldn't be achieved. So, this is just one example of many that it's very, very hard to interrupt and to treat immune responses where memory has already been established.

One thing that has worked in transplantation -- and I'm really focusing this on transplantation, because Amy asked me to talk about examples where things had worked, and I couldn't think of that many in autoimmunity, to be honest with you. And I asked Bill Sinclair [spelled phonetically] twice and got no good replies either. So I focused on transplantation, which is to do something that -- you know, I'm not suggesting this, obviously, for ERT, but just to show you what has worked, is a bone marrow transplant. So, the idea in a bone marrow transplant for solid organ transplantation is to take advantage of the fact that immune tolerance develops in the thymus, and to essentially do an

oblative, or at least a lympho-oblative preparatory regimen that eliminates all lymphocytes in the periphery, to put in donor bone marrow, and to let that donor bone marrow mature in the thymus. And in this color scheme here the recipient is yellow, and the donor is red, and we know that as cells mature in the thymus antigen reactive T cells are deleted when they see these antigens in the thymus. And if you can get some of the donor cells in the thymus that they'll be deleted as well, and what'll be left is a hole in the repertoire, but the hole should be specific for donor antigens.

And in fact -- I know the colors don't show up very well on this, but David Sachs and Ben Cosimi did that at Mass General, and they did a protocol that's pretty intensive, if you look at it. It has pre-transplant cyclophosphamide, pre-transplant rituximab, a T cell depleting antibody, which is Anti-CD2 and thymic radiation, kidney, and a donor bone marrow transplant, followed by about a year of immunosuppression, which is gradually withdrawn. And what they showed as a proof of principal was that, in fact, you could create tolerance to a solid organ across an HLA barrier, which is quite an achievement, but it wasn't all, you know, roses. So, seven patients represented by these long lines here in green were tolerant, meaning

that they took no immunosuppression for at least five years and had good renal function. Three patients, however, didn't do as well. Two lost their graft early, and one last his or her graft eventually. So it was a very bi-modal finding, and I think that it's early days in this field. I think the concept is great; it shows that this approach can work, but it also shows, as with any approach, that it's not all going to be success and, in fact, the bi-modality of this, you know, makes us all evaluate the equipoise, and will make patients think twice before they enroll in these kinds of trials.

In enzyme replacement therapy -- and I'm a novice to this field, but as I was reading through some of the papers in preparation for this, I really could think of two different situations that existed. One are the immunologically naïve patient, by which I mean there's no neutral -- no antibodies that are there, and from what I understand from, you know, my readings in preparation for this -- I'm not an expert -- is that therapy that may work -- and I know Priya will talk, I'm sure, about this -- is methotrexate rituximab and IVIG. And the presence of pre-existing antibodies, that regimen above plus bortezemib has seemed to be effective, or at least the most effective thing that I'm aware of. But I was thinking as you -- and I made

this comment earlier, I think, that there's been obviously a lot of work about immune tolerance in other fields, and fields often benefit from cross-fertilization.

So by analogy to other diseases, such as auto-immunity where antibodies are prominent -- and I'm thinking here of lupus -- things that I -- and I'm not suggesting we use these drugs -- but things that have worked, or are being investigated in lupus are drugs, like cytoxan or belimumab, which is -- which will inhibit -- potentially inhibit auto-reactive B cells. Pre-existing antibodies have also been targeted in transplantation, and I'll show you a regimen that's worked for that. But, again, it's a very complex and intensive one.

This is a bone marrow transplant regimen very similar to what I showed you in the solid organ transplant one. This one is done by Ephram Fuchs at Johns Hopkins, which has -- and this is in patients that have pre-existing antibodies against donor antigens; so a little bit different than the MGH regimen, which specifically excluded patients with pre-existing antibodies. So, here what they've done is a conditioning regimen, which I'll say is largely, we can think of, conceptually similar to the MGH one, but they've added pre-transplant plasmapheresis, low dose IVIG, and a calcineurin inhibitor, and followed that with plasmapheresis

and low dose IVG, then the bone marrow transplant, and then cytoxan.

And, what they were able to show was that they were able to reduce -- and this is the -- a way to measure donor-specific antibody -- they were able to reduce donor-specific antibody almost completely from one month pre-transplant to one month post with a much more -- essentially a non-specific effect on third-party antibodies or positive control antibodies. So, it's not to say that it can't be done, but it's an extremely intensive regiment, and I don't think it's one that anybody would think of applying for enzyme replacement therapy off the bat.

I would also point out that, although we're talking about tolerance, I've learned through my interactions with the allergy world that in the food allergy world, anyway, investigators differentiate between tolerance and desensitization. Where tolerance, to them, means no allergic responses are seen, even after a sustained period of antigen avoidance -- and that's very different than what I think of as tolerance to a solid organ where the organ's there, or to auto-immunity when the auto-antigens are there, or to ERT when you're giving enzyme replacement therapy indefinitely. They would talk about what I'm thinking of as tolerant, what we're talking about here is desensitization,

which is no response in the face of continual antigen challenge, which I would suggest seems perfectly adequate for enzyme replacement therapy, just as I would think that this would be adequate for solid organ transplantation.

The last thoughts that I would leave with, and then we can talk more about this in Q&A, is that I think it's important to treat patients early. Memory cells are hard to eliminate, and ERT is a unique opportunity to intervene before the initial exposure to antigen. The only -- organ transplantation is like that too, very different than autoimmunity. And I would suggest that you take advantage of that opportunity. We certainly don't in organ transplantation transplant somebody, let them generate an immune response, and then try and block that immune response. Combination therapies targeting B and T cells might be attractive, and I would in particular think for what works look for inspiration, or maybe for guidance in what works in antibody-mediated autoimmune diseases; that may be helpful. Low-dose continuous antigen is generally in many models less immunogenic than episodic high-dose therapy to the extent that something like that is feasible, and again, I know nothing about that. That would be something to consider as well.

I would actually make one other point that's not

on my slide, but something that I thought of today that came up -- and maybe we can discuss this, too -- is Dr. Rosenberg was recently -- did me a favor of coming to a meeting that we had in Boston to discuss tolerance trials in organ transplantation, and as she knows, one of the things that we decided at that meeting that would be helpful to the field was to try and agree on a set of guiding points or principals, the things that we should do, and to publish that as a white paper in a journal, in this case The Journal of Transplantation. And we deliberately wanted to be very noncontroversial; we wanted unanimity of everybody there so we could all say here's a pathway to move forward. And some of the things that we discussed was to try and create standardization of assays, to try and agree upon a registry of patients, to try and collect samples in a standardized way. There was a fourth one, which was to create a working group to talk to third-party payers and to Medicare about reimbursement for this. And those kinds of things can be seen as very, you know, they're little steps to say you're going to do it, and they can be hard to actually get them done, but they got everybody really motivated to not just leave the workshop, but to leave the workshop and do something, and say, "I want to work on this committee," or "I want to work on that committee," and so I don't know if

this has been discussed already, but if it hasn't, it might be something to consider. Anyway, thank you for your attention. Are we doing questions now or after? I'd be happy to take any questions.

[applause]

DR. DICKSON: I have a quick question for you. So when you talk about low dose continuous enzyme replacement therapy, are you talking about for a period of time to tolerize someone, or would that necessarily need to continue as the therapy?

DR. TURKA: It's a great question, and I don't know the answer to that. So, it might be that low doses over continuous -- over a period of time would create tolerance that would enable higher dose, intermittent therapy. I think it's something that would be worthwhile studying, but I -- so I guess I was thinking for a period of time, and then to create tolerance, but you don't know until you do it.

MALE SPEAKER: So, Larry, one of the things that was interesting in the early CTLA4Ig studies was, although in the non-human primates there was never evidence of tolerance in the transplant setting, where precursor frequency is pretty high and T cell response is strong, especially CD8s, that in those same animal studies there was

a very strong inhibition of antibody formation.

DR. TURKA: Right.

MALE SPEAKER: And given this meeting, I'm interested in your thoughts about whether there are drugs that might be better for blocking an antibody response, and we think that's a key problem here, where obviously in transplant that wasn't going to be a big -- of any big value.

DR. TURKA: No, I think it's a great point. You know, as Jeff alludes to, and I think actually it was Jeff's study that first showed this. Even though it wasn't very effective at blocking rejection, which is primarily a T cell response, it was very effective at blocking antibody responses, so it's associated with those two. And I think that it's absolutely something that should be pursued, and other reagents that act in the same general family, like reagents against icost [spelled phonetically] would be things that one would think might be just as effective, if not more so.

MALE SPEAKER: So, I was just going to add a comment about the desensitization strategy. So, normally when we're doing desensitizations it's for small molecules, like most often, you know, antibiotics, and you have to -- the patient has to be continuously exposed, you know, to the

drug, and usually if there's an interruption of more than 24 hours, we have to repeat the desensitization process, and it's a form of tachyphylaxis, because these are targeted at an IgE-mediated events. So interestingly, when we've had patients who've had IgE responses to monoclonal antibodies, we've been able to do the same desensitization protocol with, in this case, rituximab, and you would've expected based on the half-life of rituximab that you could do the desensitization, and then wait, you know, three weeks later, and then re-administer their normal dose. But we found that that's not the case, that despite the long half-life, you still have to continually desensitize them each time.

DR. TURKA: That's interesting. Well, that may be instructive.

MALE SPEAKER: I was just going to make a comment. The concept of this low dose tolerance induction of ERT is an interesting concept. The problem is sometimes in lysosomal storage disorders that patients who need it the most are the ones, like infantile onset Pompe disease, where they're rapidly deteriorating. So this idea of they need the drug, they need the enzyme replacement immediately, and giving an ineffective low dose is something that, you know, the treating physicians are loathe to do. It does beg the question, however, and people have talked about the -- with

the advent of newborn screening where you can identify the patients at birth who haven't developed the symptoms, perhaps now you can start to consider low dose induction therapies.

DR. TURKA: I think that's a great point. I'd say maybe two things. One is that we were talking about that a little bit during some of the breaks about newborn screening and the opportunity that that might potentially provide for [unintelligible] prophylactic or very, very early treatment. And your point, I think, is also a good one that, you know, there's a long list of diseases and they're not all going to be necessarily amenable to the same types of approaches, and that complicates things for sure.

Okay, great. Thank you.

Immune tolerance induction in ERT to treat infantile onset Pompe disease: Current practice

DR. DICKSON: Thank you. Our next speaker is Dr. Priya Kishnani. She is the C.L. and Su Chen Professor of Pediatrics and Chief of the Division of Medical Genetics at the Duke University School of Medicine. She's a consummate clinician investigator and a world leader in the immune -- in the enzyme replacement therapy of Pompe disease, and in the immune response to enzyme replacement therapy. Priya.

DR. KISHNANI: Thank you for the kind introduction, Patty, and I've been told I can have a little more than 25 minutes if I need it, so I'm not going to speak as fast as I normally do. So these are my financial relationships, and I will be discussing off-label and/or investigational use of rituximab and Bortezomib in my presentation. And so I really want us to go back -- and I think Dr. Rosenberg had talked about this -- but just as a quick summary of what Pompe disease is, especially for the infantile form, it is a metabolic myopathy, and it does have a continuum of clinical manifestations. And it is a deficiency of the enzyme acid alpha glucosidase. The infantile onset, which is where we are going to focus today, presents in the first few days to weeks of life, and so the idea is that it's very rapidly progressive, presents very

early. And typically they patients die because of a hypertrophic cardiomyopathy and progressive skeletal muscle weakness within the first year of life.

So in terms of treatment and success, alglucosidase alfa was approved in 2006 as the first treatment for Pompe disease, and the natural history of the disease has clearly changed. We have teenagers now with infantile Pompe disease, so despite the challenges we face in the field today, I do not want to minimize that this has been a lifesaving therapy for patients with Pompe disease. And we have these many long-term survivors, and there are many factors which we have found to affect outcome. And the high sustained antibody titers -- and I want to use each of these very carefully -- just having P titers which are high is not enough, but when you have high titers and when they are sustained over a period of time are -- have been identified to be poor prognostic factors. And it's commonly noted in what we have defined as cross-reactive immunological material negative, but also we see it in CRIM-positive cases, and we see it across the disease spectrum and not just infants, but also in adults with Pompe disease.

And so I'm not going to spend but a moment on this, because I want us to think about this very carefully.

We talk about CRIM positive and CRIM negative as though it's a black and white, but I think it's really a continuum of GA production, or CRIM as we call it, and so Dr. Rosenberg has kindly already discussed this, but I just want to say that when you have any level of GAA, or of the protein present we call it as CRIM positive, and if you have a complete lack of the protein we call it CRIM negative. And Dr. Rosenberg has talked about this, but I do want us to remember this: that even in the category of what we call CRIM positive, you could have a good or a poor clinical response to ERT, and here I should have really put the word 'typically' a poor clinical response to enzyme replacement therapy in our current definition of what we call CRIM negative.

So here when you look at a western blot -- and this is from skin biopsy, or skin fibroblast -- these are the markers and here you can see if you have any precursor band or any band that's present of the GA protein -- we call that individual CRIM positive -- and if there's an absence we call them CRIM negative. But it really depends on the method of how you're doing these assays. If you load it with a higher amount of protein, you could make someone who's CRIM negative look like CRIM positive, but these have been really done -- tested over a decade and a half at Duke

in collaboration with members from Genzyme, and I would like to attribute a lot of the success to Dr. Chen and to the Deke Shabali [spelled phonetically] in our lab, who have set a standard and done it along with mutation analysis. So the idea is looking at mutation in addition to western blot, I think, is really important when you're trying to determine or give labels of CRIM positive or CRIM negative.

So this is the kind of immune response that you have, not just to our alglucosidase alfa, but I would say to any other therapeutic protein. This is the ideal setting. You expose them and there's no problem at all; there's zero negative. The second two settings is they mount an immune response and then they show a downward trend, or they mount an immune response and then they tolerize over a period of time. And here is the problem that we face in the field of therapeutic proteins when you have the high and sustained antibody titers. And just to point out is, in our early learnings we had thought that this was in the cross-reactive immunological negative patients, but then in the early years we also recognized that we were seeing this across the disease spectrum, and it included some of the CRIM positive patients.

And so the focus of my talk today, I'm going to try and talk about what is the natural history of CRIM

negative on enzyme replacement therapy alone, and what lessons we've learned. Is there a role and a need for intervention? The second is, does early enzyme replacement therapy -- so when I say 'early,' starting at a much younger age -- does that change the immune response? And then third would be the immune modulation or the immune tolerance induction experience to date. And so this has been discussed earlier, but these were our first learnings when we separated patients by CRIM status. And when we looked at all the babies who were under six months of age and were not on invasive ventilation, and just separated them now by CRIM status, you can see that the last patient to drop off and die was about 27.1 months of age in the CRIM negative cohort, and the CRIM positive patients, yes, you did have some drop off, but the patients largely lived. And here when you look at the titers over a period of time, once again, I have experience now with what it means in infantile or in Pompe disease what these titers are, and so even at week 64 you can see increasing titers in the CRIM negative patients, and you see low to no titers in the CRIM positive patients. But if you look at the ranges very carefully, you do have some CRIM positive patients who have high titers; that is in the order of 51,200.

And so the idea is that when you have antibody

titers -- this was our very first baby that we had actually infused in the clinical trial, who unfortunately was CRIM negative. This was baby John. And if you see that at 4.2 months of age he was weak, and he at around six or seven months of age was showing a very nice clinical response to enzyme therapy, but then over time you can see at 9.5 months he's back on a ventilator, and is very moribund and barely moving his extremities. In contrast, this was from the first clinical trial, which was n=3. This was baby number three, who was CRIM positive, and fortunately for us, he was a CRIM positive who tolerized to enzyme therapy. He was started at two and a half months of age, and this was him at 12 and a half years of age. He does still have some persistent myopathy, but you can see a completely different outcome in the presence and the absence of antibody titers to these patients.

So now when we look at it, I think CRIM is just -- it's the tip of the iceberg, but we have to really look at it in terms of the antibody response and take our understanding from there, and Dr. Rosenberg already showed this. So patients who have high antibody titers do poorly, and patients with low antibody titers typically do well. Of course, there are other factors, such as the age and the stage when you start them on therapy. So by no means and by

mechanism is that this is the only end-all, be-all solution, but this is, I think, an area of therapeutic intervention where we can make a difference. And so does early treatment prevent an immune response? I really thought it was important to bring this up today, because we've been championing trying to bring some of our lysosomal storage diseases to newborn screening, and I have really championed this for Pompe disease starting in 2006, and now in 2013, Pompe disease has been put on for consideration for recommended universal -- to the recommended universal screening panel. So the question really comes about is, if you start someone in the very early stage and you're in the developing immune system, can you prevent an immune response?

And I looked at our cohort that we have, and these are all babies who are less than one month of age, and these are all CRIM negative patients. So I didn't even look at the CRIM positive patients, just looking at the CRIM negative patients. They are all under a month of age, many of them are just a couple of weeks, or a week of age. And I just want to draw your attention here to the peak titers, their weeks on ERT, and then the last titers. And here's what I'm going to point out. Patient one and patient three did poorly on enzyme therapy and died at the ages of 18 and

45 months, respectively. So one had very high titers and one had sustained intermediate titers. The second one is a patient who, I feel, we rescued, because by week four he already had a titer of 12,800. We put him on an immune suppression regimen, which I'll share with you, and he has continued to live, but I do believe that he would have gone on to mount a significant immune response. And then there were two unusual patients, patients four and five, who did not make antibody titers. So the question mark was, what was going on?

So these patients have novel mutations not previously seen in CRIM negative patients. Patient four is homozygous for a splice site mutation, and patient five is a compound heterozygous for a splice site and a frame shift mutation. And we typically have not seen this combination of mutations in our CRIM negative patients with high titers. So these are just the tip of the iceberg, and I have experience now with more than 100 CRIM negative patients, and so if we ended up with two with novel mutations who did not make titers, I'm just trying to build the case that early treatment does not prevent an immune response from occurring, and so keeping that in mind I'll go to the rest of my talk.

So now, the role of immune modulation and the

experience to date, I think of it in three settings as a clinician. One is the ideal setting when I'm able to identify the patient before I was able to start enzyme replacement therapy; so identifying them before and trying to do immune modulation alongside with enzyme replacement therapy. The second is I follow them after an early exposure to enzyme replacement therapy; so they've been exposed to some enzyme and then they've mounted an immune response, and I'm acting on this rather than waiting for months to intervene. And the third is the patient that got missed and has the high and sustained antibody titles of what we call an entrenched immune response.

So for Pompe, because it's so rapidly progressive, and because initially the CRIM assay was done on western blot, it was a challenge. There was a delay in getting the identification of these patients, and so they were already being put on enzyme therapy and then we would recognize that they're CRIM-negative. So we started looking at it by doing the correlations between the western blot and mutation analysis, and now we have a mutation database whereby if we identify a patient with infantile Pompe disease from a blood spot assay, we can turn it around in no more than two days, tell the patient or the family, or the physician, that this is likely CRIM-negative, and go ahead with that treatment

algorithm.

So in terms of, what is the distribution of these patients, I think about 75 percent are CRIM-positive and about 25 percent of these patients are CRIM-negative. So at this point, the question is, do we just initiate ERT and wait and watch if they develop titers, or do we do prophylactic immune tolerance inductions? So this is a whole separate discussion in itself, and I'm going to try and focus on the CRIM negatives, because I think the risk benefit profile goes in favor of doing something, because if we just treat with enzyme replacement therapy, the likelihood of losing these children is extremely high. So with this, we do the immune tolerance induction, which I'll get to, and then we appropriately monitor antibodies, and we do this on a monthly basis given the half-life of antibodies, and we have a quick turnaround time in collaboration with Genzyme Corporation. And then as needed, if immune modulation is needed, it's done.

So this is the data that I have up to now. We have 28 patients that have received immune tolerance induction. The vast majority of these have been in the naive setting, 22 patients, and here the CP stands for CRIM positive. And I won't be discussing this today, but just to tell you that we've started the work on CRIM positives at

this time with different induction regimens. Then is the early immune tolerance induction; so after early exposure to enzyme therapy. And then, in the entrenched immune tolerance induction setting. We have more than these but this is the data that I could capture in time for this meeting.

So in the naive setting, if we think about this -- and once again, Dr. Rosenberg has kindly discussed this -- is that the idea is that you've got therapeutic protein, and you've got the peptides and the -- you know, to the antigen-presenting cells. And then you've got this whole cascade between the T cells, the B cells, the memory B cells, and ultimately the plasma cells, which again, are short-lived, and the long-lived plasma cells, which are the final source of antibody production. So the idea is that if we prevent this cascade by the use of whatever agents -- and by no means am I trying to market the use of either rituximab or methotrexate, but that's what we have used for Pompe disease -- this was the hypothesis when we started, that if we intervened at the time of enzyme replacement therapy, or at the time of the therapeutic protein, you could probably prevent this whole cascade of events, and thus, prevent the production of antibody titers.

So in the naive setting to enzyme replacement

therapy, I want to share some of how we've built this. So you first have a diagnosis of CRIM negative, or let's say, two deleterious mutations in the GAA gene, and you do this with four rounds of rituximab, which is marked here in pink, and with nine low-dose -- nine doses of low-dose methotrexate, 0.4 milligrams per kilogram, which I think a rheumatologist or an oncologist will say is like a very, very low dose of it. And then you -- we instituted also IVIG, just because we were completely wiping out the B cells, and since these are really sick infants, this was -- it started more as a protective mechanism, but I do believe now it does play some role in the ultimate outcome of these patients.

So this is the regimen. So it's done over a five-week period and then you -- and alongside you're giving the enzyme therapy of the therapeutic protein as you would have done, and then you continue with enzyme therapy. You continue to monitor the antibodies, and if there's a need, one would have to go back and treat the patients again. And so we then looked at this and said "How would we really say when should we go back and treat?" And some of this has just been collective experience from many members in this room. The idea is that you do the immune tolerance induction concurrent with enzyme therapy. You monitor for

the antibodies, and then as a marker of B cell recovery, you look for CD19 counts. And so if you have no or low antibody titers, by which for Pompe we looked at as 6,400, and you've got CD19 recovery -- so at greater than five months, which is based on the half-life of rituximab -- you then continue to monitor the patients and the antibodies, and you see how the patient is doing.

The next setting is you've done one round of immune tolerance, or immune modulation, and now the patient is continuing to show an antibody response; and this is after B cell recovery, you are now still mounting in immune response. You need to repeat the ITI and monitor the antibodies, and see what's going on. And the other scenario is, if the antibody titers are really rapidly increasing, you may want to go back in with an agent, which would act at the level of the plasma cells, and I'll come to that in the next part of my talk.

And so this was the algorithm that we thought of, is that you monitor them; once you've seen good CD19 recovery, you not seeing antibody titers for a period of time. I actually asked Dr. Rosenberg and Dr. Turka, and Dr. Bluestone -- I'm not an immunologist -- what's usually called 'tolerance.' And I hope I can convince you that some of our patients, I do believe, are completely immune tolerant.

So this was our very first experience with these patients, and this was in collaboration with a number of physicians, and this was published in Genetics in Medicine in 2012. So this is now with a single round of the agents that I talked about, IVIG, methotrexate, and rituximab. And these are two patients. And you can see that with this short course, it led to long-term tolerance induction, and there was a good safety profile in these patients. So you can see here as -- although this looks high, this is just a titer of one in 1,600, and this is now over a period of time. This child, as I'm going to show you later on, is eight years old now. So you can see here with one round, we had CD19 recovery and no development of antibody titers, and this is now a second scenario where this occurred.

Now, in this setting where there was some exposure to the enzyme and we discovered that the patients were making antibody titers, one had a titer of one in 1,600, and one had a titer of one in 12,800. These are both -- this is an African American patient with a common mutation, which always generates high titers, and we have a natural history data set for this. And this one also you can see, within four weeks of therapy, was mounting a significant immune response. I have to say we didn't know what we were doing at the time, because these were early experience. So we

treated them with the rounds of methotrexate, rituximab, and IVIG. And then we took a small break and saw there was some CD19 recovery, and maybe a small blip in antibodies, and so continued to treat with the rituximab and the methotrexate. And this was done over a period of one to two years, because of lack of understanding of when to stop. These children have also -- they've continued to be alive and now are off all therapy, and they are immune tolerant, I would say, with CD19 recovery and no development of antibodies to the therapeutic protein.

So with this, this is the updated clinical status of the first four cases, and it was actually very nice for me to go back and update this. The current age as of May this year, is the oldest is now 8.1 years old, and we clearly know that this would not be the situation in a CRIM negative, treated with enzyme replacement therapy alone. We lost one patient who had a dilated cardiomyopathy. He was immune tolerant, but this was because of disease progression. So then I'll talk about now, the next cohort of patients of -- let me go back to safety parameters, because this is a really sick population of children, and I want to tell you that many of these have been done outpatient for the rituximab, and the methotrexate, and the IVIG. Some of the treating physicians, because these were -

- are initial experience, did the first few rounds of rituximab in-house, but then over time got quite cavalier and were doing this outpatient. The patients have done well, and some -- one of them had this mild intermittent neutropenia requiring withholding the methotrexate, and for mild viral infections. But do remember that two out of these four patients were on prolonged immune suppression because of continuous use of rituximab and of methotrexate as well.

So then we did another experience of seven CRIM negative on this same immune tolerance induction regimen. And these seven patients, I must tell you, are from around the world; some are from Canada, and a couple, I think, were from France. And so we were able to turn around the information to them, and they were treated now with the same regimen requiring one round of ITI. And so this is the natural history ERT-treated alone, or enzyme therapy treated alone, CRIM negative patients, and you can see that they have rising antibody titers over time. In contrast, when you look at these patients who have been immune modulated with this particular regimen, you can see over time that they've done extremely well from an antibody perspective.

So how does that translate really to how they are doing clinically? And here you can see that this is CRIM

negative on ERT monotherapy. I had shown this to you earlier. Invasive ventilation or death, to me, is the same end-point, and so about 27 months, the natural history cohort of CRIM-negative patients was 27 months. And here you can see the patients who are CRIM negative, who have also been treated with immune tolerance induction. I think the nice part to show you is that three who are actually on a ventilator when they started became invasive ventilator free following the immune tolerance induction protocol, telling us that the enzyme therapy had a chance to have an impact and to work.

And the same is with the left ventricular mass index of the heart. So the heart is considered a very responsive organ and patients on enzyme therapy for Pompe do very well from a cardiac perspective, even if they don't have a skeletal muscle response. But I really want to point out one thing. If I did not use this natural history cohort of patients, you know where you can see that this goes up slightly, it's still a dramatic response, because it's not the upward trend that you would see with LVMI. And so even when I say when we use end points, we have got to understand how we are using our end points. If I had used this, I would say this is a responder in a CRIM-negative patient being treated with ERT alone, because it's a downward trend,

and it's stabilization. But now when I compare it to someone who's started at the much higher left ventricular mass index, but pre-treated with enzyme replacement therapy and immune tolerance induction, you can see that we are able to bring them into the normal range. So I want to bring home this point that our understanding of end points is as good as we want to understand and what we want to make out of them.

And so now this is our collective experience of using this particular regimen with 17 patients that I've been able to gather data on, and so this is the comparison. And you can see that we've really, really changed the natural course of CRIM-negative patients who've been treated with ERT plus immune modulation. So now the safety of the regimen for at least these seven patients. I do want to point out that the current age of this second cohort of patients of seven, we've lost three of these babies at 1.3 to two-and-a-half years of age. And this was, again, because of disease progression; it was not at the time of immune toleration induction. They were tolerized, will continue to receive enzyme therapy. A couple of them died due to pneumonia later on in life. But what I also want to show you is that they've gone on to receive their regular immunizations. They've had a recovery, and when you look at

tetanus toxoid and diphtheria, you know, they are mounting an antibody response to tell us that these immunizations are working.

And this is the -- for the information on these patients, this doesn't show very clearly here, but you can see the CD19, they've all shown recovery. Two of these patients actually required a second round of immune tolerance induction, because I was kind of nervous when I saw the titers of 6,400. I could have waited, but the risk of them having an entrenched immune response, to me, was way more than trying to go with the second round of immune modulation, which may have not been necessary, but was done. And all of them have had vaccinations to date and are doing quite well. The other path that I want to point out is these were all done in an outpatient setting.

So now, let's look at the immune tolerance induction protocol for patients with the high-sustained antibody titers, or what we call an established or an entrenched immune response setting. And I want to talk about this, because as I showed you, this was the kind of scenarios that we face. And so when we are in this high and sustained setting, the real idea would be to intervene with the immune modulation prior, but sometimes, we're stuck with the situation in the clinical world where the patient has

high and sustained antibody titers. And this is the kind of difference that you see. This is the CRIM-positive patient who did not make antibody titers, and this is a CRIM-positive patient who made high antibody titers. And I'm going to try and share this story of how much I learned from this little boy to the extreme right.

So when we looked at this cascade of events, what I started to recognize as a clinician was we were targeting the T cells and B cells, and rituximab acts at the level of where you have CD20, but the plasma cells, many of them, do not have CD20, and so the idea is that if we do not target these cells in the entrenched immune response setting, these long-lived plasma cells, which are there for years and continue to spew antibodies, were naturally going to make a clinical difference. So we were very keen to try and identify an agent that would work in this particular setting. And so when I started doing a pub-med search, I came across bortezomib or Velcade, which is used for multiple myeloma, which we know is a plasma cell tumor. It's a proteasome inhibitor, and it targets the mature antibody producing plasma cells. So it was FDA approved, and as a result of which, you know, they could be off-labeled use of this particular agent in a clinical setting. So bortezomib, as I had seen in the literature, had been

used clinically in kidney transplant rejection with success, where they were able to bring down the donor-specific antigen antibody production, and also some success pre-clinically in the lupus nephritis mouse model, and in other auto-immune diseases.

So this is the little one that I showed you that video of very briefly. So he was diagnosed at age five months. He was a CRIM-positive infantile Pompe baby, and started therapy at 5.4 months with alglucosidase alpha. He had initial improvement in the first six months of therapy, he had new motor milestones, did not require ventilated support, his left ventricular mass had come down from a base line of 262, and had come down to about 135 grams per meter squared. So when we think of the upper limits of normal, its 64 grams per meter squared. So still there were some enlargement of the heart, but he was heading in the right direction. And then he started to show a clinical decline, despite enzyme therapy, and his titers had peaked up to about 204,800. So by age 14 months -- so nine months now into enzyme therapy -- his titers were about 102,400. He was now diffusely hypotonic, he was requiring BiPAP following an upper respiratory infection, and at age 18 months, he became invasive ventilated dependent. And now, interestingly, if you see his left ventricular mass, it had

gone up from that improvement of 135 to about 360 grams per meter squared. And he had also outflow tract obstruction, which is really telling us that even the cardiac response was failing despite enzyme replacement therapy. He also required g-tube placement, because of severe dysphasia.

And at this time, the agents that were in consideration, or in use based on previous experience, had been cyclophosphamide and rituximab, and so he was -- this was attempted in this situation, and there was really no improvement for this young baby. He was unable to move his arms or legs, and his voluntary motor activity was just limited now to eye movements. So the physician, Dr. Kabori [spelled phonetically] on the West Coast, had given me a call, and we had just started looking at bortezomib and done some pre-clinical work with it, and it was a very ethically challenging situation, because I was trying to tell her what to do, but it was in a child who was now dying. And so we spoke to the ethics committee, and the mother and the father of this child, who was supposed to be at this meeting but unfortunately was unable to come, and it was really a heroic attempt and I have to thank Dr. Kabori and the team there for taking this challenge on.

So we started with bortezomib and we added it now to this regimen. We removed, of course, the

cyclophosphamide and we said "Let's try what we've used now with rituximab, methotrexate, and IVIG, but let's add bortezomib to this regimen, and if truly it's acting at the level of plasma cells, and if we can prevent new antibody production, we may have some success." So I'll try and show you how this worked, but pretty much, we had a decline in titers over a short period of time from 204,800 to one in 100, and the left ventricular mass index now improved from 360 to 72 grams per meter squared. This child was now starting to move his upper and lower extremities. There was some improvement in the myopathic facies. He had a decrease in his vent settings initially, had less oxygen requirements, and now increase in time off the ventilator.

So this is how it worked. Initially, with the cyclophosphamide and the rituximab, you can see that there was not really much impact. And then after the first round of bortezomib, which includes four doses of bortezomib at the dose of 1.3 milligrams per meter squared, you can see that we were seeing a downward trend, but it was still pretty high at 25,600. And this is just showing you the antibody titers. And then, if you look at the next one, with the second round of bortezomib, the titers had come down to about 6,400, and then after three rounds of bortezomib, it was down to one in 100, and we were also

seeing a very dramatic improvement in the left ventricular mass index for this particular baby.

So with that, I have to tell you that this paper took more than two years to -- it started with a single case report. It was not publishable. This is anecdotal experience, so when people talk about publishing this, I know the hardships with this. And so we were -- in the meanwhile, we went on to do two more cases, and I didn't go into discussing those, but this was our first patient that I talked to you about. And then we did it in patient number two, who also had a titer now of 819,200, a CRIM-negative baby. And you can see, this is at -- until the time of publication, his titers had come down after the addition of bortezomib to the regiment to 6,400. So this really requires combination therapy. I'm not saying, it's bortezomib alone, but you've got to hit at the various levels, the T-cells, the B-cells, as well as the plasma cells. And then this was patient number three, and this was, at that time, 3,200.

And so with that, what I want to point out to you is we were finally able to publish this experience, but I want to go back to what we've learned from our data. So this is CRIM negative and CRIM positive; so these are high-titer patients, treated with enzyme replacement therapy

alone, and these are their antibody titers increasing. These are what we call the low-titer patients, so who were showing a downward trend, and then these are the patients from our very first experience with the use of immune modulation, and this was with rituximab, cyclophosphamide, plasmapheresis, and high -- frequent infusions of the alglucosidase alfa at five times per week. And you can see that we were really not doing anything for these patients in terms of reducing the antibody titers. In fact, one of them went on to develop a nephrotic syndrome, which was reversible, so when we stopped the immune modulation -- or stopped the enzyme replacement therapy back to the therapeutic doses, we were able to reverse that event. And then these are our patients now who were treated with the bortezomib, methotrexate, IVIG, and rituximab regimen. So clearly, I think, it had made some difference.

So I want to also give you an update on these three cases, because at that point, when we had published, it was immune suppression. They were still receiving rituximab not at the same frequency, but they were still receiving it. And this is where they are right now -- this is in years -- 9.2 years, 7.3 years, and six and a half years. So these are our children that had been started, and this is where they are today. And I also want to really say

is that the current status is two out of the three are really off all immune tolerance induction agents for more than a year and a half now, and do not have an antibody response to the alglucosidase alfa. In this particular situation, the physician has been kind of nervous, but is stretching it out, the rituximab and the methotrexate to further out, but as you can see, the titers have come down beautifully. And at this, in terms of the serious adverse events that have been non-related to the immune tolerance induction that these patients have received.

So I really want to end by -- with a few videos, but also by saying that in our growing care of these patients with the lysosomal storage diseases, it is a team that makes it work. And I think at the center of it are our parents, our patients, and the care coordinators. And I think one of our very invaluable members of this team is an immunologist, because without their support, I think it's hard to do this in this field. And I just want to show you how I feel. We're trying to change the natural history now of sacrum negative patients. So this is that historic baby, and I always remember Baby John, because I -- this was the baby we lost to therapy, and this was a commitment that we at Duke had made to the family that we would make an impact, and we would try and make a difference for these patients.

And so here is one of our patients treated with enzyme replacement therapy, and now at age three and a half years -- this is Dr. Raymond Wong's [spelled phonetically] patient -- and as you can see, she is mobile and ambulating, and eating by mouth. And here is -- in the high-sustained setting, these are more challenging of how do you really look for endpoints, but this is the little fellow. As he had started enzyme therapy, you can see what a nice response he had shown. He was sitting, which you don't see in the natural history of infantile Pompe disease. This was him as he started to mount an immune response, getting floppy, was now being G-tube fed, requiring BiPAP help. And then this is him now at six and a half years of age. He's off the ventilator for several hours of the day, and he's actually started school. He's starting kindergarten. So to say that -- we've not cured this child, by no means have we been able to completely reverse it, but we've clearly given him life and really good quality of life. I've met him. I don't typically go to patient's homes, but this was a separate situation, because it was on the West Coast and the mother had invited me. And he reads, you know, through the use of computer devices, a very bright young fellow.

And so in summary, I would say that the natural cause of these patients, you know, with deleterious

mutations or what we call CRIM negative on ERT monotherapy shows that patients mount an immune response and do not do well, and that early treatment, at least in our hands, up to now, does not really protect against the development of high titers. I also would like to add that this CRIM determination ideally should be done by both western blot, as well as mutation analysis, or can be restricted to mutation analysis, but the role of understanding the genotype, I think, is really, really critical as we move ahead, because it allows for the understanding of the immune response, it will allow us for epitope mapping, and for moving ahead in this field. And also, that immune modulation in the CRIM-negative setting -- naive setting, so in the naive setting has shown success with good safety and efficacy in a large cohort of patients. And patients with high-sustained titers can be tolerized with enzyme therapy and immune modulation, but requires prolonged immune suppression. And to me, the risk-benefit ratio, at least in this clinical scenario, favors the use of immune modulation therapies in the naive setting. And as the safety profile appears good, there is consideration for use in CRIM-positive cases as well, and we are starting to do this work -- or we have started this work at Duke already.

And so I really want to acknowledge the members

from my division, many of the members from the Immune Tolerance Network -- and I should have actually thanked Larry Turka, and Jeff as well, for all their support through the years, from the FDA, from Anne Pariser and Amy Rosenberg, in particular. Funding sources, without the industry support from Genzyme Corporation, I don't think we could've really moved ahead; and clearly from the Lysosomal Disease Network, which has allowed us to build and grow on our experience, and most importantly our patients and their families. Thank you.

[applause]

FEMALE SPEAKER: And this is like [inaudible]

DR. DICKSON: We have time for a few questions.

MALE SPEAKER: Very nice talk, Priya. Given the fact that we've said today that it's very hard to eliminate the memory cells once they're formed, and given this very remarkable safety profile, low incidence of adverse events in these patients -- pretty sick patients, too, CRIM negative -- if you turn your attention to the late-onset, CRIM-positive patients, is there any reason not to give a very short course of immune tolerance induction prior to starting ERT right now? Or do you think for the smaller number of CRIM-positive patients that are going to go on to develop sustained high-titer antibodies, you can rescue them

with bortezomib? How do you weigh those two things?

DR. KISHNANI: You actually are helping me with my own -- I personally believe that we should be doing this, and we have started this work in the CRIM-positive infants, as well as the adults. We're trying to even use a more attenuated regimen, because we're trying to reduce the risk even further and see if this would work. But absolutely, I think that it makes sense, at least in my mind, to immune modulate at the outset rather than this whole case of antibody titers, and then trying to rescue these patients, and then trying to find groups of physicians, because not everyone can come to tertiary care centers, and many of them are uncomfortable with these kind of regimens. So I would say yes.

MALE SPEAKER: Just to take the devil's advocate for a second, what about the long-term effects of these medications? What do we know about these five or 10 years down the line? Because what you're proposing are patients who probably -- a number have never need any immune modulation. You want to treat everybody to prevent those 10 to 15 percent of CRIM-positive patients, who I presume, are going to get antibodies. Is that correct?

DR. KISHNANI: So I'm actually putting -- going to put this question back to you for a moment, is we don't know

what antibodies are doing at the time when we've started with enzyme replacement therapy. We don't know what it is, if you're CDER-negative from the outset, whether your response to therapy -- because it's not just the antibody titers. I do believe that there's a whole cascade of inflammatory response that's going on, and I think that we may have an opportunity to learn by doing this. And so I don't think it's just a 10 to 15 percent who would develop. That's the high and sustained. But we do know that there is seroconversion going on in the bulk of therapeutic proteins, and in many of them, they're not really tolerizing. They maintain lower titers, or whatever we call lower titers, but there still is an area under the curve which we don't know what it's doing.

So to me, I think that if you can get a regimen that's even easier and simpler, and even more safe, ideally this is at least what I would like to do as a treating clinician. And we're not using rituximab. In fact, to me, it's the contrary: that once you're stuck with an immune response then you're using longer term duration of these B-cell agents, which could have potential for side effects rather than in a controlled setting and in a shorter term.

MALE SPEAKER: But I presume you're making the assumption that these drugs have no long-term side effects.

DR. KISHNANI: So I don't work for either of them, but what I'm saying is I think there's plenty of experience with these agents over the long haul where they have been used, and so --

MALE SPEAKER: What's the 'long haul' mean?

DR. KISHNANI: Rituximab has been on the market for how many years? I mean, Amy, it's been 10? Yeah, and that's been -- and it's been used for longer durations. I mean, maybe Jeff or Amy, one of you could answer to this. I mean, here we're speaking about a short course, you know, versus the other challenge. And by no means am I saying it has to be done on every patient, every disease. It has to be disease-specific and dependent on the kind of immune response.

MALE SPEAKER: Yeah, I mean, somebody should -- you know, can look up and look at the official data, probably here at the FDA better than anybody else, but there have been very rare cases of any long-term problems with Rituxan --

MALE SPEAKER: But again, the long term is --

MALE SPEAKER: -- [inaudible] cases of PML out of 10,000 or so, but very, very, very few long term side effects. Infections have been quite minimal in general. It's turned out to be a pretty safe drug.

MALE SPEAKER: In the population it was used. I think it's important to know what --

MALE SPEAKER: Well, what's interesting about the population is, is it's gotten more and more diverse. It started out as a cancer drug, but now is used in a variety of autoimmune diseases, as well as similar safety.

MALE SPEAKER: Okay. So Priya, going back to your question, I think it's important to do this in a setting of a very controlled environment before a recommendation would go out so that everybody with Pompe needs immune suppression, because if that happens and all the other lysosomal storage disorders will have that same sort of -- people will start doing immune suppression in all sorts of different manners --

DR. KISHNANI: [affirmative]

MALE SPEAKER: -- without the controlled environment.

DR. KISHNANI: Right. And I think we've tried to be very careful and very judicious about how we are doing it, and that's why we've taken the stepwise approach, Joe. It's not like, you know, we're saying to do it and all. We're taking it one step at a time to try and, you know, get a better understanding in the field.

MR. DE CLARO: I'm Angelo DeCarlo with Division of

Hematology Products with Office of Hematology and Oncology here in FDA. I'm the medical officer team leader for Velcade and rituximab. And regarding questions on long-term safety of these drugs, I mean, our experience is mainly in the oncology setting, wherein these drugs have been used for years in some patients. We do recognize that our current -- the current prescribing information for both drugs are lacking information for experience in the pediatric patients, so I think your approach of carefully collecting safety information in your population is an excellent approach to characterize the safety in your population that you're studying. Thank you.

DR. KISHNANI: Thank you.

DR. PARISER: Thank you very much, Dr. Kishnani, and thank you for all these great questions. We will come back to this during the panel discussion time.

MR. NIKOLOV: I just want -- so my name is Nicolay Nikolov and I'm a medical officer in the Division of Pulmonary, Allergy, and Rheumatology Products, the one that oversees the rheumatological indications, which is rheumatoid arthritis and ANCA vasculitis, ANCA-associated vasculitis. I just wanted to give a little bit of background on the use of rituximab, at least in pediatrics in our indications, and it's essentially nonexistent. It's

very limited. We have been concerned in general with the effects in the pediatric population of potential side effects of rituximab in this patient population, including agammaglobulinemia, hypogammaglobulinemia, and risk of serious infections, and that was one of the reasons we decided not to require pediatric studies in the juvenile idiopathic arthritis. With respect to long-term efficacy, we have no existing data in, you know, in kids. In general, when we talk about lifesaving or life-prolonging treatments, like immune tolerizing regimens for enzyme replacement therapy, the risk-benefit in this situation is completely different than what we consider for the rheumatologic indications.

DR. PARISER: Thank you very much, both Angelo and Nikolai [spelled phonetically]. I hope we can have you come back to the microphone during the panel discussion. Thank you.

Non-cytotoxic, non-immunosuppressing approach to tolerance induction during ERT treatment

DR. PARISER: Okay. I'd like to move on to our next speaker. It's Dr. Jeanine Utz. She is the -- an adjunct assistant professor in the Department of Experiment and Clinical Pharmacology, and also works in the clinic in the Department of Pediatrics at the University of Minnesota. Most of her research is in lysosomal storage disorders, and she's going to be presenting another case of non-cytotoxic, non-immunosuppressing approach to tolerance induction during ERT treatment. So thank you.

DR. UTZ: Is this my -- how do I move this down? With my finger or -- oops. Uh-oh. That was the screen.

[laughter]

DR. UTZ: Okay.

DR. PARISER: Okay. This is forward, reverse. Okay.

DR. UTZ: Should I move the screen back down or --

DR. PARISER: That's fine.

DR. UTZ: Oh, okay. For some reason, when I'm pushing on this button, it's not moving. Oh, there we go. Okay. So, okay. Okay. I got to the first slide.

DR. PARISER: [inaudible]

DR. UTZ: I was pressing this to go forward.

DR. PARISER: [inaudible]

DR. UTZ: Okay. I pressed that one so I won't press that one. Here we go. Okay. Yes. Maybe it'll work now.

DR. PARISER: Okay.

DR. UTZ: Okay. So intravenous enzyme replacement therapy is FDA-approved for seven of the lysosomal diseases, and it has shown the ability to stabilize or slow many of the disease processes associated with these diseases. Today, we're talking about antibodies to enzyme replacement therapy, or ERT, and in particular concern, the inhibiting antibodies, which may develop in some patients. We sometimes call them inhibiting or neutralizing, but they can hinder the efficacy of ERT by several mechanisms. Again, we've discussed much of this today, but to summarize here, neutralizing or inhibiting antibodies can reduce enzyme stability. They can result -- they can cause enzyme degradation in the blood stream. They can also cause an antibody-mediated blockade of cell receptor uptake of ERT. They can cause retargeting of the ERT -- of the M6P glycosylated ERT to macrophages where subsequently it could be destroyed or cause other downstream effects. And they can also result or cause intercellular misrouting of ERT.

These are some agents used to commonly -- that

have been commonly used over the last two to three decades for immune tolerization regimens, and Dr. Turka has gone over this, as well as Dr. Rosenberg. But I highlighted in red that most of the agents that have been used and have shown efficacy do seem to have some sort of inhibition of T cell activity or B cell activity, or both. It's important to know on the bortezomib, as Priya was talking about, it actually is -- of all these agents, it is the only one that really produces a sustained inhibition of long-lived plasma cells that may be causing part of this immune tolerance or neutralization of the enzyme. Side effects of these agents long-term could potentially be concerning. It looks like the safety profile for rituximab has been good over the last 10 to 12 years, since it came to market, and methotrexate has been used for decades as an autoimmune -- for treatment of autoimmune diseases with relatively good safety profile. But I do appreciate the concern about safety in these -- using these agents in these patients, and we still have to see how that plays out long-term.

The rapidity of the immune tolerizing regimen can be increased, as Priya and others have discussed, by using combination regimens of these agents. And Priya discussed the methotrexate, rituximab, and bortezomib. If we add intravenous immunoglobulin to that, or IVIG, we can also

increase the rapidity of the immune tolerization. And how does IVIG work? We, you know, we aren't exactly sure how it's working in every patient, but these are some proposed mechanisms: Blockade of Fc receptors and macrophages, which could prevent phagocytosis or circulating antigen taint with antibodies. It may provide anti-idiotypic antibodies that can neutralize pathogenic autoantibodies. This may be more something that we'd look at in the autoimmune disease. Down-regulation of endogenous immunoglobulin, complement absorption, enhancement of suppressor cells, and inhibition of lymphocyte proliferation.

Are there risks with IVIG? We view this drug as having very little, very low risk in terms of long-term safety, but there are some risks that I put on this slide, just to bring to the attention of those who may prescribe it, or are considering immune tolerance regimens. Patients, of course, can have infusion reactions. Sometimes with IVIG, they have a delayed infusion reaction. Those of you who used it may have seen this, sort of flu-like symptoms. But some more serious side effects that have been associated with IVIG, and -- although very rarely, are thrombotic events, acute renal failure. And the thrombotic effects and acute renal failure seem to be associated with the speed of the infusion and the osmolality of the drug. Acute renal

failure also appears to be associated with the sucrose stabilizer. So the only -- the reason why these toxicities are so important is you can actually choose products that will minimize this risk if you're using IVIG. And looking at the discussion of immune tolerance regimens we're having today, most likely, whichever regimen you choose, will have IVIG in it.

So dose-intensive immune-tolerizing regimens. I'm calling -- I guess I named this description for this slide as dose-intensive. I'm really referring to something that Dr. Turka mentioned also, where you may give the drug more frequently, you may give it at higher doses. This is the antigen, the drug, the antigen, or both, and this has been tried for immune tolerization regimens. These regimens may be slower acting. They have the advantage of allowing the patient to receive the therapeutic agent while they're going through immune tolerization. They're often combined with IVIG to sort of expedite the immune-tolerization process. One example is in hemophilia in patients that have inhibitors to infuse Factor VIII.

One of the risks of doing the higher dose or the more intense dosing is, well, there're case reports, very few, a rare risk is nephrotic syndrome, and Priya mentioned to this in a Pompe patient who did develop nephrotic

syndrome On a higher dosing regimen. It could've been related to the high dose of ERT, the antigen, possibly the other agents [inaudible] that we don't know for sure. But the patient did fully recover after removal of the more intensive therapy. And one of the factors that may play into this risk for nephrotic syndrome is just the size of the molecule. So this slide just shows the molecular mass of the ERTs that are on the market, and as you can see, they're -- this is in Daltons. Elosulfase is 110,000 Daltons, aglucosidase is 105,000 Daltons. These are very high molecular weight drugs. The average drug we take by mouth usually has a molecular mass of about 200, and I showed miglustat is a small molecule of therapy at the bottom, FDA-approved for treatment of Gaucher disease in oral therapy. There're only 219 Daltons and that is very common.

So when we infuse these ERT drugs, we are giving a very high amount of molecular mass in terms of the drug, and this was speculated to be the cause of the nephrotic syndrome cases that were reported in Factor IX patients that had inhibitors to the Factor IX -- recombinant Factor IX. Factor VIII, when it is used in intensive therapies for patients with hemophilia A or developing neutralizing antibodies, has not been shown to cause nephrotic syndrome.

And when you look at the difference between the two regimens, they're both using a dose of 100 unit per kilogram many times -- it's a common dose -- but the actual amount of drug in terms of the molecular mass the patient receives is 50 times higher in the patients receiving Factor IX. Another important consideration is where the drug goes. For patients with hemophilia A, their factor goes into the intravascular space. Patients with hemophilia B, the Factor IX infused goes intravascular and extravascular. So these may play roles in some of these risks.

So that was the background, and I will just share with you a patient that we have been treating with immune tolerization regimen with Hunter syndrome. He was diagnosed at 14 months old. His plasma iduronate sulfatase was zero. His pretreatment quantitated urine GAG was nine times the upper limit of normal; so, elevated. The urine heparan sulfate was elevated at 26.9 percent. We also did a liver needle biopsy, which showed finely vacuolated hepatocytes and Kupffer cells consistent with mucopolysaccharidosis. This is our patient taken about a month ago, a picture of him. We did have a conundrum with him. We cannot find his Hunter disease mutation, and this is an X-linked disease, but we cannot find the mutation. We sequenced the exomes at both Cincinnati Children's and the University of Minnesota,

could not find the mutation; sequenced the promoter region, that was normal; sequenced the SUMF1 gene for multiple sulfatase deficiency, that was normal. So we do not know this patient's mutation.

But this is showing in terms of outcomes, how do we measure outcomes to immune tolerance regimens? How do we measure outcomes to ERT in general? And in the MPS conditions, this can be challenging. Usually, you know, we look at their urine GAG and that's pretty clear in terms of diagnostic criteria. And you could see on this slide the pretreatment quantitative urine GAG was nine times the upper limit of normal, and that went down after starting ERT. So after one month of ERT, it was decreased about -- by about five-fold down to 1.6 times the upper limit of normal. We had difficulty measuring other clinical outcomes in this patient, and the reason is he had quite significant cognitive impairment that was worsening. So clinical trials for many of the ERTs for MPS conditions are really looking at urine GAG and six-minute or 12-minute walk test, range of motion, maybe pulmonary function, or at least these are parameters that come out in the literature, either in clinical trials or shortly thereafter. In his case, we could not give him instructions to do a six-minute walk test. He would not follow them. He could not follow

instructions very adequately for doing a pulmonary function testing. He would not do the range of motion for us. He was rather belligerent. So we have urine GAG. So I don't know the answer to this question of having just one biomarker in one that has some problems with it in terms of, you know, fine tuning of your therapies but that's where it is.

So the urine GAG pretreatment was nine times the upper limit of normal, and I should say the reason I'm saying upper limit of normal is urine GAG, the normal reference ranges change with age, so I'm referring to how it relates to the age reference range. One month after beginning treatment, it significantly reduced to what we would consider pretty close to a normal range. And then for two years, from 1.5 year -- or for the next year, 1.5 years to 2.5 years after being on weekly therapy, it just kind of hung in there in the two to four-fold times the upper limit of normal range. We were satisfied with that, that's what we've seen with other patients, so we were okay with that.

Three and a half years after starting therapy, the urine GAG jumped and we thought, well, maybe this is just a lab error, maybe, you know, we'll keep measuring it, but it did sustain a higher level. And he also showed clinical worsening that was quite remarkable, and the speed of the

worsening was accentuated. He had respiratory tract infections that were chronic at this point. And when it first started, the ERT, the respiratory tract infections had diminished, he seemed to be infection-free from longer periods of time, but he had chronic recurrent respiratory tract infections that occurred at this three and a half year mark and thereafter. He also had chronic rhinorrhea. He had fevers with this many -- oftentimes, worsening dysmorphic features. In fact, he kind of looked to us like he'd never been treated the way -- from physical appearance, and he was complaining of head pain. He couldn't put a sentence together, but he would point at his head and say "Ow." And lumbar puncture showed increased cerebrospinal fluid pressure. A ventriculoperitoneal shunt was placed.

And then we did ask Shire, can we measure neutralizing antibodies? Do you think he's making some sort of inhibitor or neutralizing antibody? And they said, "Yes. Here's how you do it," they gave us the instructions. So we measured the levels, and for about eight months here, these are -- this is the percent inhibition. It's essentially 100 percent inhibition of his enzyme, but these are in vitro tests, so of course, they may be -- not exactly what's happening in vivo, but we got most of the results back at the same time. So we sent all these tests in, but

then you wait four to six months to get the results back. So we waited for that. During that time, we thought, "What can we do for an immune tolerization regimen?" And we seriously considered the regimen Priya is using, and maybe that would've been a great regimen for him. We haven't tried it on yet. You know, it is a backup plan for us, but we were looking at his condition with these chronic infections and he also has valvular disease, heart valve disease from his MPS II condition. We don't know if there could be increased risk of colonization in the valves, if we were suppressing T cells. We just were a little concerned about the infectious risk.

So we did try doing something that is more of a dose-intense regimen; however, with the low dose. Now, ideally what we wanted to do was give him continual enzyme 24 hours a day. We were not able to set that up. We maybe could've put it in a pump and pumped it in 24 hours a day, but he was, again, sort of belligerent. He'd pull on his port line, and he just probably would not have cooperated. We did end up giving him a dose-intensive regimen that -- where he got ERT every day, actually twice a day, in the morning and at night, and we had them infuse it through an elastomeric device at home. So the parents were trained on how to add the drug to the elastomeric device and just -- we

would lock it onto his port and infuse it over an hour in the morning and an hour at night, twice daily. His total weekly dose was 1 milligram per kilogram per week. The FDA-approved dose is 0.58 milligrams per kilogram per week, so this was not twice the normal dose, but kind of getting close to twice the normal dose. And we also gave him intravenous immunoglobulin 200 milligrams per kilogram weekly. We kind of came up with this idea based on the history of immune tolerance regimens used for patients with hemophilia who have inhibitors.

So here's what happened with his regimen. The first four -- first four points in there are his percent inhibition prior to starting this regimen. Then we started the regimen and we did see a drop in the neutralization. So this is the percent neutralization of the anti-ERT antibodies. The shaded area is what Shire Lab had considered a negligible amount of neutralization. So as you can see on this -- on the latter half of the graph, much of that time, he's in what they consider negligible. I'm actually not sure how they come up with that number, and they may want to speak to that, but at one point, you could see on the -- on the -- about three-fourths of the way through the graph, he actually -- his neutralizing antibody percent inhibition was zero. That did increase sharply and

we don't know why, but it's -- one thing that changed at that time is we went from giving the drug twice daily to once daily, so the same total daily dose, but we thought while he's at zero, let's make it more convenient for the parents and go up to once daily dosing. We did see a sharp rise in inhibiting antibodies at that time, but we kept him on that once daily regimen along with IVIG, we continued to see it come back down.

This is just his overall antibody levels during this time. They don't exactly mimic the neutralizing anti -- percent inhibition, so there is a difference there. And in red at the bottom, you'll see where we -- his -- after 18 months of this regimen, his urine GAG was in the normal range. I should clarify that that last level in red was done by the CPC method, whereas the other ones were done by the DMB method. The pretreatment level was CPC, so there's some variation in the results you get back depending on which method you use, but overall, it's still clearly a response of urine GAG.

This again is -- this chart is a chart of his urine GAG levels. The top one is straight urine GAG. It doesn't take into account his age, so it's just looking at where it was. On the left-hand side is his urine GAG after he was stabilized on therapy. I don't have the pretreatment

GAG on this graph but it was -- it would be way off the charts, nine times the upper limit of normal. So here he is on the left-hand side with a urine GAG that's roughly two to three times the upper limit of normal, and then it went up to six times the upper limit of normal, that peak in the middle, and then came back down. The chart below is somewhat similar but it is adjusted for the normal reference range for the age, so you see it looks a little bit different when you adjust urine GAG values for his age.

In conclusion, this regimen which we are calling non-cit [spelled phonetically] regimen right now, non-cytotoxic, non-immunotoxic immune tolerance regimen using increased ERT dose intensity, combined with IVIG did reduce the neutralizing antibodies to the iduronate sulfatase from 100 percent down to negative levels for a child with Hunter syndrome. And it may be considered as an alternative regimen or possibly an appropriate regimen for patients whose underlying disease may result in a higher risk of infection or certain types of infection that you're trying to watch out for, or you want -- you're -- and you're comfortable with achieving immune tolerance over a longer period of time. You know, clearly, this would not be appropriate in an infant with a CRIM -- with Pompe disease with a CRIM-negative status. And that concludes. Thanks.

[applause]

DR. DICKSON: Thank you.

DR. UTZ: Okay.

Experience with ERT: Parent's perspective

DR. DICKSON: Okay. In the interest of time, we'll be asking everyone to save questions until the panel discussion. So our next speaker is Mr. Steve Holland. Steve is president of the National MPS Society and an MPS I parent. He has been involved in enzyme replacement therapy for MPS I since the beginning. And he has been a great inspiration to many, including myself, because it was about 10 years ago when Steve first invited me to have breakfast with him and his family and literally sucked me into the MPS world and I have never left. So Steve.

MR. HOLLAND: And we're happy about that, we're happy about that. Hello, everyone. Thanks to the FDA and to NORD for inviting me to speak today, but I guess I have to say now for something totally different, okay? You've been looking at graphs, you've been looking at charts, you've been looking at numbers, and this is just to remind you that behind every data point is a face and a name and I'm going to share with you a little bit about those faces and names. As Patty said, I'm the father of three incredibly brave MPS I children with the attenuated form of MPS I or Hurler-Scheie syndrome, and also president of the MPS Society which is a support group for all of the MPS and ML syndromes in which we have about 800 members.

Today, I'm going to talk about a parent's perspective on enzyme replacement therapy and I hope that you see our family is just a representative family of all the families that have rare diseases, and I hope you -- when you hear our story, you'll think, "You know, I could've been that guy. I think I want to help him and help him a lot." So with that, I'll introduce you to my family here. There's my wife, Amy, and I, and then our three children, from left to right, Laney [spelled phonetically], Maddison [spelled phonetically], and Spencer [spelled phonetically], and this picture was taken back in 2006 and I'm just especially fond of that one. But to understand our story, you sort of have to know the beginning of our story.

So it starts way back in the 1980's. You see there in the middle, there's Amy and I. We were high school sweethearts. We went on our first date when I was 16, she was 14. If she was here, she would say, "Now, you need to say that it was a group date because my mother would not let me go on a car date at 14, so get that out of the way." We dated all through high school and then into college. You see us there in the band. And then in 1986, we were married after I graduated from college with an accounting degree. So you can see our genetic fate was sealed at a quite an early age. We did what most young families do if I can get

the slide to change here. There we go. We started having kiddos. So despite the best-laid five-year plan that I had, Spencer came along at three and a half years in 1989, and then Madison surprised us just 18 months later in 1991, and then Laney joined the family two years later, 1993. So we were doing all the normal things that normal young families do, just enjoying life, and thinking of the future.

But things started to change. So these were not big things at the time. It's only when we look back on them that we can see a pattern, and see what they really were. You know, one thing, the kids were always sick, it seemed like. They had ear tubes, all of them, a couple of sets. They had some other issues that were a little bit different. You know, one was they couldn't raise their arms above their head. You know, we took them to the pediatrician and said, "Look, they can't raise their arms above their head. What's going on?" And he said, "Ah, don't worry about it. It's probably something genetic." We said okay and we went on living our lives. But then Maddie presented with anisocoria in 1994, which was uneven pupils. Now, we knew enough to know that that wasn't quite normal, and that led us into the world of specialists, and we saw an ophthalmologist and then a neurologist. And you know, the neurologist did MRI, and he came back and he said, "Well,

I'm pleased to tell you that your child does not have a brain tumor." So we said, "Well, thank you very much." But he said, "You know, tell me a little bit about Maddie. Is there anything unusual about her?"

And we said, "Well, you know, she can't put her arms above her head, but it's nothing to worry about, because it's probably just genetic." Well, he was a little bit brighter than our pediatrician and he knew not to let that go. So two months later, all three of our children were diagnosed with a terminal genetic disease, so our little slice of heaven here on earth was changed, and changed forever. We had the diagnosis of MPS I, and we knew it's a terminal disease. You know, we really didn't know at the time about this autosomal recessive stuff, so you know, in hindsight, it was one out of four, one out of four, one out of four. And now you all are smart doctors, so you know that's one out of 64, so that's less than a 2 percent chance that that would happen if we had known, you know, of course we were carriers, which we did not. So the way we explain that, you know, to our friends -- at the time they quoted for the attenuated form of MPS is being 1 out of 500,000 births. So we said that there're 1.5 million people out there that we've covered births for just from our family.

So I do want to point out though that that's the

way our family was diagnosed. That's not typical of most MPS families. So most MPS families, the kids showed a defect early on, they missed developmental milestones, they learn skills, they start to not learn those skills, they start to lose skills, and that leads them to a diagnosis. So we had a very quick diagnosis. Often, there's not a quick diagnosis. So it was speedy, it was done over the telephone. I'll always remember that telephone call. I don't recommend that for the geneticists in here. Also, just because the doctors didn't know much about it, we were presented with the worst case scenario, which was the full-blown severe Hurler scenario. You know, we immediately took the kids to a geneticist to examine Spencer who was the oldest because we only had the results back from Maddie, our middle child. And she said, "Oh, he doesn't have it based on the physical exam." But unfortunately, when the testing came back, they all did. You know, at the time, the BMT rate, you know, they were losing about half the kids that they transplanted, so we knew that probably wasn't the right thing to do at that point in time. And you know, we actually, you know, obviously grieved for the loss of all the dreams and hopes we had for our kids and we reached out to the MPS Society and that's sort of where I got sucked in and just like I said earlier.

So we started our new life as post diagnosis. You know, we began our annual visits with all our doctors and went to MPS conferences. And then in 1997, we had heard the BMT rates had increased, had improved a little bit, so we went up to the University of Minnesota and was considering actually doing a transplant. They looked at our kids and said, "Oh, you'll probably have lots of matches" just looking at us. And the way it turned out, there were no matches. It was not an option for us at all. But when that door was shut, just a few months later, in '97, we heard about Emil Kakkis' ERT therapy that was coming up and we enrolled Spencer in that. So one thing I was going to point out when going to all the doctors' offices, you can imagine we had three kids in there plus I have sort of a crazy wife. So some of the doctors, you know, if they made us wait in there too long, then they were really in for a treat, because, you know, the crowd mentality would set in and we would start singing Christmas songs and it'd get louder and louder and louder until someone would come see us. Plus, there was maybe a balloon or two you might have with the gloves, I think, for the doctor when they came in.

Anyway, Spencer was in the phase I/II study. I'll get this to change here in a minute. There we go. For the ERT trial in 1998, he's the third one from the left. He was

the fourth patient to be in that study. And then after that is sort of what I call the dark period because that is when Spencer started getting better. Just, you know, immediately, he started having more energy, he started not napping during the day, we could just see, you know, more range of motion. But then we had the girls and they were getting worse, so it really tugged at our parents' hearts', you know, strings that we had one child getting better and the others were getting worse. That actually created some opportunities for us to be on national TV related to our situation. But three long years later, the girls were able to enroll in the phase III study and you may recognize the doctor there a few years ago, Dr. Muenzer. So we traveled weekly up to North Carolina and eventually moved up there for the rest of that study. But one thing about that, you know, we pushed the girls in wheelchairs through the airport for the first time we were there. And then by week two, Laney pushed Maddie and she continued to push her the rest of the time. So we knew there was a placebo in that one of the girls would be on and the one would be off. It was very clear by the second week to us that Laney was receiving the drug and Maddie wasn't. And sure enough, when it was un-blinded, that turned out to be that way.

Then in 2003, we were thrilled to be able to

participate in a panel hearing for Aldurazyme. This building wasn't here yet but we met in a hotel in the area and it was such a great thing to be able to hear that panel recommend yes, yes, yes, yes, because we knew that had that not been approved, there was little chance that any of the other MPS's would have ever been approved. And I will say the second best day was this past November when we were in this room and the families talked about the Morquio -- the Vimizim and Morquio IVA and the same thing happened. It was just a thrill to ourselves. So what -- after it was approved, we went on and did our infusions in the hospital. And of course, you know, being parents, we always want something better, so soon after that, we moved to home infusions. And the girls have had the same nurse for 12 years. They have a little chick party and friends and family come by and they really make it a fun time, so we're really pleased about that.

So just a few words about allergic reactions. You know, our kids have always had a lot of allergic reactions. From the earliest days I can remember, we went to an allergist and they tested them on their back, like they do, and they prick them and all that, and they're saying, you know, "This is a 3 and this is a 2" and I'm like "What about this one?" And they said, "Well, that's the control." And

I said, "Well, it's supposed to be blank." And they're like, "Yeah, I know. I'm not sure why that happened." But anyway, their skin, I don't know, something about them reacting. They did a lot. The girls were allergic to most all of the antibiotics. Eventually, we had Maddie tested and actually tolerized the penicillin or maybe it was the other word you used earlier, but you know, being a mixing agent and she was able to take that that we're pleased about. But related to the Aldurazyme, Spencer on his fourth treatment, so we're still in L.A. at this study, doing the trial, and he had this stomach reaction and the smooth muscle contractions and that's just something -- we were used to hives and stuff but not this big doubling over pain. And you can imagine, you know, here all this thought and preparation and work had gotten to be in the study, and all of a sudden, the fourth time, he's got this huge reaction and we're wondering, you know, what's going to happen. Well, luckily, they were able to put him on steroids. He did gain 25 percent of his body weight over the next few months, but still, he was able to continue taking the drug, which we were thrilled about, and we've never stopped since then.

You know, the kids have been -- generally have had hives. The stomach pain happened more than once, you know,

flushing, wheezing. Spencer, especially, had to have some breathing treatments but it was always able to be managed, so we saw already Dr. Burton had talked about this earlier with steroids. We used IV versus oral Benadryl which worked for him, lengthened infusions. The doctors explained to us that they always had many options for us and that we never had to worry about that. And you know, nowadays, if something -- if someone has the hives, it's because they forgot to take a premed or something and it's easily taken care of.

So just in, you know, this is actually the 20-year anniversary of my children being diagnosed so just reflecting back on our time, you know, as a parent of a special needs children, I can tell you that my risk tolerance for potential benefit is greater than the general public. My children's disease is degenerative and terminal, and without intervention, I know what will happen. That gives me the courage to accept risk that has the potential for changing that equation. You know, had those 10 families in the phase I/II study for Aldurazyme chosen not to accept that risk or, even worst, not to be given that opportunity, think of the thousands of kids who have never benefitted from ERT. This increased risk tolerance is also evidenced by the vast majority of the severe Hurler families that

accept the significant risk of bone marrow transplantation to stabilize their children's cognition. I believe ERT has stabilized most of the physical impacts of my children's disease. Their quality of life is significantly better than it would've been otherwise and I believe it has been life extending, and that is why we must find a way to get it to everyone that needs it, including those with these allergic reactions and these titers. Thank you.

[applause]

DR. DICKSON: Thank you, Steve. And our last speaker in this section is Melissa Hogan who is an MPS II parent and with experience in immune modulation and tolerance for enzyme replacement therapy.

MS. HOGAN: And I'll clarify that at a little bit. I'm actually reading a statement from another parent. I think we were going to have a parent here who had experience with immune modulation and they were unable to make it. As an aside question, something we haven't addressed in the panel, and I hope we will, my son has been receiving intrathecal enzyme replacement for three and a half years, and I think that presents an additional challenge with respect to antibodies and titers, monitoring, and therapies. So I'll read the statement. Hi. My name is Jamie Fowler [spelled phonetically] and I offered to provide comments on our experience with immune tolerance induction since I was unable to attend the workshop due to my son's medical appointments. My friend, Melissa Hogan, who was already serving as a panelist, offered to read my comments for me.

My son, Jack, is six and a half years old and has Hunter syndrome or MPS II. He has a full gene deletion and his body makes no enzymes so he is CRIM negative. He started ERT after diagnosis at the age of 18 months and no

one ever mentioned the concept of antibodies, his CRIM status, or immune tolerance. While he had positive physical effects from ERT early on, these effects waned as time went on. Around age four in 2011, Jack started to present with uneven heart rate and headaches during infusions increasing in duration and frequency. By the beginning of 2012, we started to see rashes and a flushed face, and later hives and itching by the end of his infusions. We treated it with a slower rate and added more Benadryl to the premed regimen. Around the same time, we noticed distention of his belly, increased lethargy resulting in weight gain, and shortness of breath. GAG testing showed them as significantly high for someone receiving ERT for over two years. Our geneticist at the time felt like the only course of action available was to increase premeds and slow the infusion rate, so no testing was done.

In the spring of 2012, we brought up our concerns with Dr. Barbara Burton who was following Jack on a yearly basis. She ordered antibody testing and also requested to review Jack's HOS study data, which included clinical symptoms and labs to date. Once we received lab results, we learned that Jack's antibodies had steadily increased and he was IgG and IgE positive. We researched and spoke with a number of physicians, specifically some who were considered

experts in MPS. Some were staunched in their opinion that one, genotype did not necessarily make one more at risk for antibodies or reactions; and/or two, that antibodies did not relate to reactions or effectiveness of drug. However, Dr. Burton listened to our concern and investigated further. She presented Jack's lab findings to a team of immunologists which we understand unanimously responded that Jack was not responding to enzyme. We read extensively on immune tolerance induction to learn and assess better for ourselves. We became convinced of his need for immune tolerance induction in order to restore the effectiveness of ERT. Our local physician in Denver refused to perform immune tolerance induction. Thus, in July 2012, we left our jobs, sold our house, and moved our family to another state to live near a physician who was willing to develop and implement a protocol of ITI for Jack.

The protocol was developed with consultation among many of the experts on ITI, some of whom are here today. It took longer than we expected, but our son's antibodies are now normalized. However, there are several salient points I'd like to make about our experience. Regarding antibodies provider perspectives. Many providers who diagnose and treat our children have no understanding of the relationship between genotype, ERT, antibodies, and reactions. In fact,

many disavow such relationship. Many also are unaware of the potential use of immune tolerance induction and parents are left floundering for themselves with their only guidance being research articles and other parents. Thus, some published conclusions developed from today's discussion will be helpful to families in their efforts to educate their physicians, nurses, and insurance companies that they deal with.

Regarding immune tolerance induction timing. Given what we've learned and our experience with the difficulty of normalizing antibodies after the titers are high, it is our opinion that at least in Hunter syndrome, caregivers should be made aware of their child's CRIM status prior to beginning ERT. The implications of that status, even for CRIM positive patients, should be explained in terms of antibody development and reactions and that at least for CRIM-negative patients, prophylactic ITI should be recommended.

Regarding antibody testing process. The process of antibody testing was quite frustrating as the only party who could test for antibodies, and this is still the case, although it's now outsourced, is the pharmaceutical company who makes our ERT. Until recently, for almost everyone, antibody tests were batched and families often didn't

receive results for six months or more. Ours were run STAT given our immune tolerance regimen. This has made it difficult for families to make therapeutic decisions for their children. I would recommend that the FDA require the availability of prompt and efficient antibody testing following FDA approval of any new or current enzyme replacement therapy.

Regarding insurance reimbursement. Immune tolerance induction is quite a new area for insurance companies. Several drugs in our protocol were denied but we luckily had a very knowledgeable and committed provider who followed up with our insurance company. Many families are not so lucky and I know of at least one circumstance where a child clearly needs ITI but it has been denied by insurance. Again, published conclusions and/or guidance could be helpful for insurance companies to better understand the background of and necessity for ITI in certain circumstances.

Regarding post-ITI. While the goal of ITI is antibody normalization, we cannot forget that the finish line does not always create smooth sailing. Infusions for us are still very long, double the original time period, require premeds, and involve reactions. Whether that's because ITI was therapeutic as opposed to prophylactic or

because in CRIM-negative MPS II patients that will always be the case, we don't know. But creating immune tolerance does not fully solve the problem that many ERTs may still pose immunogenic challenges for groups of patients. We need to be vigilant in assessing immunogenicity in both CRIM-negative and CRIM-positive and in as many applicable genotypes as possible and making sure that information is shared with patients, caregivers, and providers.

Regarding implications for ERT clinical trials. Because antibodies are more likely to develop, at least as I've read, in CRIM-negative patients which are the severe mutations or deletions at least in Hunter syndrome who are more likely to be cognitively impaired, it is of utter importance that these severe mutations/deletions be included in clinical trials for any ERT products, whether intravenous, intrathecal, or otherwise, and that antibody information be accumulated and shared with patients, given that it has significant safety, effectiveness, and therapeutic decision-making implications. Thank you.

[applause]

DR. PARISER: Thank you very much, to all our speakers. We've fallen a little bit off our agenda timeline so what we'd like to propose is that we take a quick 10-minute break here and then have everybody come back and

we'll have one continuous panel and question and answer session. So it is 2:26, so if we could all be back in our chairs at 2:36 ready to go.

[off the record]

Panel Discussion

DR. DICKSON: Can everyone please return to their seats for the panel discussion? And can I ask the speakers, if they're not already up here, to come take a seat at the panel -- what shall I call this -- the panel counter of attention -- the panel desk.

Can everyone go ahead and get their seats? We're going to get started. Can all the speakers from session two please have a seat at the panelist desk area, up here in the front.

Okay. So welcome back to the panel discussion for session two on the Role of Immune Tolerance Induction and Enzyme Replacement Therapy. We had some lovely talks in this session and I think it raised a lot questions and a lot of thoughts -- a lot of discussion points and things we might want to talk about. Some of the points I'd like to raise for you are the -- or from the letter, from Jack Fowler's mother, talking about how having been through the experience of having the Enzyme Replacement Therapy, not having immune modulation ahead of time, needing immune modulation and then her appeal to all of us to consider doing this, a priority.

And so I think that, that's really what we need to address, which is basically point one of the panel

discussion points that are listed here on the screen; discuss which patients receiving ERT should be considered for Immune Tolerance Induction and the criteria that should be used to define the most appropriate candidates. And I want to read the rest of the discussion points along the side of that because I think they all kind of flow together.

Discuss whether decisions on the risk benefit of Immune Tolerance Induction could be made prior to demonstration of clinical efficacy. If so discuss studies that should be considered in early stages of development, to enable risk benefit assessment. Discuss the methods of assessing the efficacy of immune tolerance induction, this is a key issue of outcomes and how do we study whether we're having an impact on things that maybe aren't so immediately felt. And discuss whether extrapolation of efficacy and safety of immune tolerance induction is possible between lysosomal diseases and from other indications. And also, I would like to ask -- say, that we are going to be losing our immunologist of Dr. Jeffrey Bluestone and Dr. Larry Turka have to leave early and so if there are discussion points relevant to -- well, immunology, which is probably quite a few discussion points relevant to immunology, ask that they be made now, and we ask if the two of you have any comments

that you like to make now?

DR. BLUESTONE: Let me just make a couple of quick and rather glib comments. Since its being recorded it'll be for posterity. So, to me in general having listened and looked at the data that this fundamentally seems like a no-brainer to me. That as an immunologist who understands a little bit about the immune system, it's very clear from Priya's work and others that one could make a big difference in at least a subset of these individuals by providing a tolerogenic therapy at the -- before therapy and it would be, I think for those patients, have an enormously positive impact.

So, how you get from here to there I think is the challenge for both the companies and the physicians to feel comfortable with how do you get from here to there, but it would seem to me to be unethical not to be thinking about leasing a subset of these patients treating with some of these drugs now that are been well tested in a variety of patient populations and shown to be safe, and in this setting have an appearance of being efficacious.

So how do you get from here to there? I think very clear immune monitoring is going to be essential and it's going to be very important to look at these anti-bodies very early on, after treatment begins and the CRIM positive

individuals, but in the CRIM negative individuals I don't see any reason why one would consider treating prophylactically with some of these drugs, because I think such a high percentage of these individuals are going to be auto-antibody positive and can't be a good thing, from my perspective.

And then the last point that I'll make is, just that, after the induction of tolerance, hopefully in a large group of these patients really investing in looking at what the outcome of the treatment was, really trying to look at the immune system to see how it's been modified, to see whether in fact there is an active regulation by the immune system. Again, since we'll probably pay off a lot of dividends later on, when one tries to develop assays to predict who might or might or not respond to these enzymes and so, I -- to me again, just to say what I said at the beginning, it just seems almost imperative that some very active, pro-active approach to try to incorporate immune modulating drugs early on in this intervention is essential for the patients that are getting these drugs.

DR. TURKA: So, I pretty much agree, in fact, I entirely agree with everything that Jeff said. I mean, I would add that many of these drugs, and Jeff's alluded to this infinitive in patients for years, in some cases maybe

up to a decade or more, and there have been thousands or tens of thousands patients treated with them. I think those are the right drugs to use, I don't think this is necessarily the best setting to use drugs that have had approval more recently or in which a smaller number of patients have been so treated, but the, you know, prophylactic treatment in the CRIM negative patients with drugs that have been -- for which there is an extensive track record of usage, seems to me the ideal setting in which to start.

Obviously, just because, you know, thousands or sometimes tens of thousands of patients have been treated for a decade doesn't mean that there are no side-effects and that there's no risk to this, but it does give you a sense of at least what a risk benefit ratio might look like. And I agree anti-bodies over the long term, I don't think will be good, and my guess would be, by perhaps extension to other diseases, that the longer therapies like enzyme replacement therapy are available the longer patients, and this is a good thing obviously, will be alive. And the more potential there will be to see the downsides of anti-body production over time, so, I would argue rather than wait for those things to happen, it would be good to start treating.

DR. DICKSON: You have a question in the back.

MALE SPEAKER: Yes, at least one speaker mentioned the potential of infections as being a -- something to look out for, in this world where, you know, potentially infantile and juvenile and adult on-site diseases are being treated with ERT, you have potentially complex medical cases where there are -- could be more than one condition, effecting the individual. Is this going to be a case by case basis where you're looking at whether, you know, off-setting the benefits and risk of therapy versus some kind of --

DR. ROSENBERG: Can you repeat the questions?

DR. DICKSON: I'm sorry.

MALE SPEAKER: So my question was, I guess, have -- in the cases that have been put on ERT, are there side effects such as infections which sort of or -- risk that outweigh the benefits of the therapy.

DR. DICKSON: Are there -- the question is; are there life-threatening, or very severe, infections that have occurred with the patients who have received immune-modulation or immune-suppressive therapy for enzyme replacement therapy, is that correct?

MALE SPEAKER: That's correct.

AMY ROSENBERG: So, I would certainly differ to Priya who has the most experience, but it seems to me the

Pompe, infantile Pompe is the worst case scenario, because here you have kids who are -- whose muscles are very weak, they can't clear secretions, if anybody's going to get, you know, bronchitis and pneumonia and really a severe infection, I would think it would be these kids and yet they've done really remarkably well, so Priya, I would welcome your comments.

DR. KISHNANI: I would agree with what you say Amy. And there are situations when you've got to make clinical judgment calls, so although you have a protocol, it is in the clinical real-world setting, so if someone has a low white-cell count you can skip that dose of methotrexate and come back at it later, but the idea is it's -- you know, you follow a certain group of drugs because you think they're acting by a certain mechanism of action and the key is to do it at the time of enzyme replacement therapy because, once you've escaped that period of time, the downstream of when to stop and how long to go is really challenging.

So in this real-world, I mean, honestly I'm one physician in the U.S. and these are children and babies from around the world that are doing it, and we're not seeing, you know, multitudes of deaths or anything from this. They're really sick kids and they are still handing this

quite well. Often, you know, they skip a dose of methotrexate, or they postpone a dose of rituximab, but it still has worked.

MALE SPEAKER: All right, thanks. My concern is just over the long-course, could there be a potential --

DR. DICKSON: The question is over the long-course will there be a potential risk? The question was over the long course, over the long-term any potential risk?

DR. BLUESTONE: So I think, you know, and it's probably worthwhile doing a full-literature review and doing it -- I spent a little bit of time looking at my computer while the question was asked earlier, and for rituxamab plus methotrexate, and remember in most of those trials the rituximab is being given continuously, or multi-dosing, which was not done here, there was very limited amount of adverse -- severe adverse events, either metabolic or infectious. As I mentioned before, there were a couple of cases of a disease-caused PML, there have been 14,000 human years of experience now with this drug, and there are a couple of cases of a virus infection so, I think that this drug -- and again it's for everybody to look it up, this drug is being, this combination of methotrexate and Rituxan has been pretty well tested now, and has been, you know, considered, I think, a fairly safe therapy. It's not to say

there aren't examples of infections, there are, but they are very limited and if you look on the label it'll show you exactly Hepatitis B, for people who are exposed is one that's come up as well. But I think overall the safety profile of this particular combination has been very good.

DR. DICKSON: Anne wants to make a quick comment.

DR. PARISER: Yeah, I wonder if this would be a good time to ask Angelo and Nikolai, if they wouldn't mind coming back to the microphone and continuing their previous comments because I think what was brought up -- because there's very limited experience in pediatrics and I wonder if you couldn't expand on that just a little bit.

MR. DECLARA: Hi, sorry, Angelo DeCarlo with Oncology, my background is in adult hematology/oncology. So the -- these two -- Rituxan and velcade -- Rituxan was approved in 1997 and velcade was approved in 2003, so certainly we've had more than a decade experience with both and the safety profile is correct rates for the indications for which they are approved for. So I think, really, we're going long-term safety, I mean, it's difficult to extrapolate, for example, the oncology population to this population, because of -- we are -- there are differences in the underlying disease, we're dealing with neo-plastic lymphocytes and neo-plastic plasma cells versus, these are

not.

And typically we give these drugs in combination with other cytotoxic chemotherapy drugs at higher doses that have been described. So, I think it's difficult to extrapolate the safety profile. Nevertheless, I think, mean, the effects of the adverse reactions being described, mainly infections, and mild suppression are consistent with what's been seen in the oncology literature.

DR. BLUESTONE: Isn't it true now, that Rituxan has also been approved for ANCA-positive vasculitis?

MALE SPEAKER: That's correct.

MALE SPEAKER: Air [spelled phonetically] is known as well right?

MR. DECLARA: So the experience with ANCA-positive vasculitis is a bit more limited. We have fewer patients exposed, our most -- most of our safety exposure database comes from patients with rheumatoid arthritis, which is the other approved indication, and it's in patients who are fairly sick, they have failed all the therapies including biologics, and in that patient population the safety profile was deemed acceptable, particularly with respect to serious infections.

Rituximab is labeled, and you can go through label essentially, whatever's important and we know about it, it's

in the label. Before I was referring to the pediatric -- the experience with rituximab exposure in pediatric patients, which is very limited at least, from the rheumatology side, and we were really concerned with more short-term toxicities not necessarily longer-term toxicity but also with the development of the immune system and whether giving rituximab to young patients might impact the development of the immune system, and whether they may develop an adequate repertoire of long-lived plasma cells that they may need throughout life. And that's why in the patients with juvenile idiopathic arthritis, there are alternative treatments the risk benefit profile was not that favorable to require sponsors to these studies. So we have very limited experience, short and long-term in pediatric patients with rheumatologic indications.

I think in the case of this really severe spectrum of Pompes disease, this might be a completely different ballgame, or the risk, even with the PML might be justified. That's up for discussion. The question comes whether this population can be defined enough to us as the risk benefit in this patient group. Because there was -- you know, the other end of the spectrum where the phenotype might not be as severe and documenting risk for benefit might take longer and might need larger studies.

DR. KISHNANI: Could I just make one comment in terms of the infections risk? When we had initially started this, we had just added the IVIG, you know, to it, and it has really helped in terms of the safety profile, and prevented the secondary infections when they are so immune suppressed. That's been really helpful. And I think the second point is that this regiment is just one that we've used, it doesn't have to -- it could be a first generation regiment it's just opening it up to industry and to many academicians in this room to think of other ways to do this, so that we can get this really safe protocol. I think the idea here is if we can even come to an agreement or to a conclusion that, yes, it is really important to do this immune modulation at the time of enzyme therapy in a certain category of patients as a starting place. I think that's a win-win for all of us here.

FEMALE SPEAKER: Charlie? Charlie --

MALE SPEAKER: I was just going to emphasize the difference in the two regiments. One is the immune tolerance induction regiment for patients who have never started the I.T. What Priya's talking about right, Priya's you're talking about a very short regiment of five months and then that's it, you take the patients off five -- excuse me, five weeks, okay. And so there's been a lot of talk

here about what do we know about the long-term effects of Rituxan and methotrexate and other diseases. This is a whole different ballgame here when we're talking about a very short treatment. So, I imagine the longer you get away from the five weeks of treatment there, the less concerned you are about the long-term effects of methotrexate, Rituxan.

Now it's slightly different situation when you're in a situation with sustained high-titer anti-bodies and you're trying to chase these anti-bodies down and you're still working out how long patients need to be on that before you withdraw them from treatment. Then the considerations for long-term treatment of Rituxan and methotrexate and perhaps the addition of Pertuzumab [spelled phonetically] and others to that, you get some pretty heroic regiments when that's different. But for the immunoprophylaxis prior to treating ERT it seems pretty safe here, the five weeks, and so that's the one that we ought to concentrate on.

DR. KISHNANI: Thank you so much for clarifying. And it's a duration of five weeks, it's not daily therapy for five weeks. It's really four doses of rituximab and nine low-dose methotrexate .4 mg per kg instead of the 2-4 mg per kg which is used as the methatroxate dose, correct me

if I'm wrong, for a much longer period in oncology. So it's really pretty attenuated what we're talking about here.

FEMALE SPEAKER: Rekha?

DR. ABICHANDANI: This is Rekha Abichandani, a couple of questions. Number one is does it matter at what age this immune -- this profalactic immune regiment is started when you're talking about very young kids verses slightly older because it might be different when they are diagnosed, you know, we talked a little bit about newborns [unintelligible] earlier. So does it matter if these -- if one were to start immune-tolerizing really young kids, verses immune-tolerizing somebody who's like three or four years old, that's one question. And the second thing is that, you know, what are the panels thoughts about -- is it like one size fits all, is every disease like Pompe or is -- what do you do for diseases that are less severe manifestations, is a prophylactic immune tolerance regiment warranted? How, do we kind of sort of that out, when do we start? And before demonstration of clinical benefit, I think some diseases it's harder, I think, to make a -- I mean, Pompe the effect is so dramatic it's much easier to see, but I think that some other diseases it might be harder to….

DR. DICKSON: I mean, I want to address that

quickly, because I mean, I think, we're looking at Pompe disease because there's a clinical impact that's clear, we're looking at MPS-1 because of the animal data, but really for the other ones -- I mean, if it were not for the MPS-1 animal data we wouldn't have any idea, and we still don't know for sure, what impact these anti-bodies have on MPS-1 patients and so, I agree this is a matter for big discussion. We talked a lot about doing this in select patients, but who are the select patients? I mean, I think it's very clear who some of them are, but there may be quite a few more, or potentially even, almost all patients receiving ERT.

DR. KISHNANI: Rekha, I'll try and address your question of the very young versus the older, I mean, typically what we've done it in as young a two-week old, we've done it as young as in one-week old, and I think the oldest that we've done it, right now, is in a ten-month old in the naïve setting. And then of course, in the entrenched immune response it's been in older so, to me I think it's more challenging when you're doing it and, not just by the age, but even the stage of the disease. You know, if you've got a healthy infant verses a really sick infant, it doesn't matter if it's a ten-month old or a three-day old, I think it's more, in that situation, what I would be more worried.

And then I think the other point is that, we have to really see, to me, the immune system is the immune system and so it's a therapeutic protein and you're -- you know, you're getting a response, so this class of agents, anything acting at the level of T cells and B cells to me, is what's important. It doesn't matter what that specific agent is, it's just that this has been tried and there's been some success.

DR. TURKA: If I can maybe add to that and, I agree, it seems to me that the risk of whatever regiment is, is going to be in general, probably independent of which ERT setting it's put in, it's the risk. There may be, you know, a little bit of fine tuning here and there, but it's going to be the risk. The benefit obviously will vary, depending upon the disease, the course of the disease, how severely effected somebody is, so at least you can in thinking about it, one variable is not a variable, it's a constant; it's the risk of a protocol.

And then where the benefit will be, and where it will be seen or where a disease comes out in sort of a good-risk benefit ratio, will depend upon which ERT you're talking about. It also seems, just hearing this, and this is, you know, I'm newish to this area but, I agree with what

Priya's saying, I think it's been a terrific demonstration of principle by her, Amy, Jeanine, and to a few others, that this can be done.

So, it doesn't seem to me that, you know, the group today could or should necessarily decide this is the protocol, this is how we're going to do it. It's more a principal that it can be done, there are combinations of agents that work against plasma cells, B cells and T cells that can be effected, and now it seems to me the decision is does -- do -- does the community have the desire to push this forward? If so, how do you get to consensus on which diseases, what regiment, and where do you start?

DR. DICKSON: Question?

FEMALE SPEAKER: Yeah, continuing on his point, so the regiment for naïve setting is actually just five weeks, right? If the patients are already identified as anti-- high anti-body titers, is the regiment longer? And so, there -- I think that's an important point, because if you start in the naïve setting, you're actually getting away with five weeks of dosing the long-term effects are actually not so long-term. Whereas if you go, you wait, you have high anti-body titers, they're actually exposing the patients to a lot more medications which might include bortezomib at this point, and a longer duration of therapy

and actually the risk-benefit ratio is actually tilting even to the worse, if you wait longer.

FEMALE SPEAKER: Is that correct?

DR. HOGAN: I think you are correct, and I would say too, that the toll of the up and down battle on patients, both health-wise and just looking at -- questioning the efficacy and trying to go back and get those under control later is such a vast difference from the prospect of doing a five week prophylactic treatment, that families who have now watched individual families go through this, you know, are horrified at the prospect of having to later come back and try to get under control high-sustaining anti-bodies.

FEMALE SPEAKER: And just to touch upon the previous topic where we were trying to see if different assays can become paired against each other and can we pick down to a titer as being some kind of a trigger for how treatment should be done, I am coming from the assay end of the world, and I've looked at a number of these assays and the titer can change depending on the dose that is being given to the patient -- within the same patient over time. So, without a clear understanding of what the assay is and what the titer really means, I would hate for anyone to pick something to a titer value and make decisions based on that,

and I just think it's worth mentioning at this point that I think the titer should be a guide to see how one patient is performing over time, within that patient, but really beyond that it's not of very high value.

MR. WILLIS: Chuck Willis, Minneapolis [spelled phonetically]. I wanted to put a point on the considerations of different diseases and how this relates to some others. I think when you think about MPS-1 and MPS-2, remember that these diseases often present with multiple -- a history of multiple ear infections, sometimes pneumonias, so before they're diagnosed, they've already demonstrated they have a high risk for infections. So it is a different situation than Pompe disease.

I guess the other thing I want to think about is for MPS-1 it's almost irrelevant when we talk about Hurler syndrome. Every child in the U.S., you're not going to say that maybe a little bit boldly, but I think that every patient to the patient goes through an ITA protocol that's called hematopoietic stem cell transplant. They get typically nowadays a couple doses of aldurazyme, then they go to complete myeloablation, and they get transplanted with a donor who's making normal enzymes.

So, I think that's a specific and different, unique situation. So, I think there are situations where we

definitely will have -- with better examples of when anti-bodies are impacting the effective drug, and I think it still might be among those that have more attenuated forms. We also have to think ahead to Sanfilippos syndrome, I think Charlie Richards will tell you published data; says that six out of 25, or more than 20 percent of patients with Sanfilippos syndrome has severe disorder basing, making no effective protein.

So, there are some things to learn, it may not be exactly the examples we're thinking about and then finally, one question, this is an area of major ignorance for me, if we mandate an ITI protocol for patients with any severe disease or CRIM-negative disease, can we extrapolate from hemophilia? Does every hemophiliac, as soon as they're diagnosed, have an ITA protocol? Does that apply to factor nine as well as to factor eight, where factor eight disease, they're unlikely to evolve anti-bodies. Can we learn from that experience?

DR. BLUESTONE: So, all great comments and great questions, I'd ask you a question, maybe you could -- so in my experience, bone-marrow transplant is not an innocuous treatment, and in fact is, makes patients, if anything, very susceptible to infections, especially in an aloe besides graft versus host. So the community has made that an

acceptable enzyme replacement therapy, for a patient population that has a lot of earaches before diagnosis.

So I'm interested in your thinking about why in this case, actually potentially using tolerogenic therapies to make the enzyme replacement as robust as bone-marrow transplant is something that seems far more dangerous to you than a bone marrow transplant.

MR. MINNEAPOLIS: Well, I didn't say it was more dangerous, but I will say a transplant is more efficacious, and it's kind of unique to this set of diseases. Enzyme replacement therapy does not cross the blood-brain barrier, even though I showed data to the opposite that was 20-fold normal therapeutic

DR. DICKSON: Can everyone please return to their seats for the panel discussion? And can I ask the speakers, if they're not already up here, to come take a seat at the panel -- what shall I call this-- the panel counter of attention -- the panel desk. Can everyone go ahead and get their seats? We're going to get started. Can all the speakers from session two please have a seat at the panelist desk area, up here in the front.

Okay. So welcome back to the panel discussion for session two on the Role of Immune Tolerance Induction and Enzyme Replacement Therapy. We had some lovely talks in

this session and I think it raised a lot questions and a lot of thoughts -- a lot of discussion points and things we might want to talk about. Some of the points I'd like to raise for you are the -- or from the letter, from Jack Fowler's mother, talking about how having been through the experience of having the Enzyme Replacement Therapy, not having immune modulation ahead of time, needing immune modulation and then her appeal to all of us to consider doing this, a priority. And so I think that, that's really what we need to address, which is basically point one of the panel discussion points that are listed here on the screen; discuss which patients receiving ERT should be considered for Immune Tolerance Induction and the criteria that should be used to define the most appropriate candidates. And I want to read the rest of the discussion points along the side of that because I think they all kind of flow together.

Discuss whether decisions on the risk benefit of Immune Tolerance Induction could be made prior to demonstration of clinical efficacy. If so discuss studies that should be considered in early stages of development, to enable risk benefit assessment. Discuss the methods of assessing the efficacy of immune tolerance induction, this is a key issue of outcomes and how do we study whether we're

having an impact on things that maybe aren't so immediately felt. And discuss whether extrapolation of efficacy and safety of immune tolerance induction is possible between Lysosomal Diseases and from other indications. And also, I would like to ask -- say, that we are going to be losing our immunologist of Dr. Jeffrey Bluestone and Dr. Larry Turka have to leave early and so if there are discussion points relevant to -- well, immunology, which is probably quite a few discussion points relevant to immunology, ask that they be made now, and we ask if the two of you have any comments that you like to make now?

DR. BLUESTONE: Let me just make a couple of quick and rather glib comments. Since its being recorded it'll be for posterity. So, to me in general having listened and looked at the data that this fundamentally seems like a no-brainer to me. That as an immunologist who understands a little bit about the immune system, it's very clear from Priya's work and others that one could make a big difference in at least a subset of these individuals by providing a toleragenic [spelled phonetically] therapy at the -- before therapy and it would be, I think for those patients, have an enormously positive impact.

So, how you get from here to there I think is the challenge for both the companies and the physicians to feel

comfortable with how do you get from here to there, but it would seem to me to be unethical not to be thinking about leasing a subset of these patients treating with some of these drugs now that are been well tested in a variety of patient populations and shown to be safe, and in this setting have an appearance of being efficacious. So how do you get from here to there? I think very clear immune monitoring is going to be essential and it's going to be very important to look at these anti-bodies very early on, after treatment begins and the CRIM positive individuals, but in the CRIM negative individuals I don't see any reason why one would consider treating prophylactically with some of these drugs, because I think such a high percentage of these individuals are going to be auto-anti-body positive and can't be a good thing, from my perspective.

And then the last point that I'll make is, just that, after the induction of tolerance, hopefully in a large group of these patients really investing in looking at what the outcome of the treatment was, really trying to look at the immune system to see how it's been modified, to see whether in fact there is an active regulation by the immune system. Again, since we'll probably pay off a lot of dividends later on, when one tries to develop assays to predict who might or might or not respond to these enzymes

and so, I -- to me again, just to say what I said at the beginning, it just seems almost imperative that some very active, pro-active approach to try to incorporate immune modulating drugs early on in this intervention is essential for the patients that are getting these drugs.

DR. TURKA: So, I pretty much agree, in fact, I entirely agree with everything that Jeff said. I mean, I would add that many of these drugs, and Jeff's alluded to this infinitive in patients for years, in some cases maybe up to a decade or more, and there have been thousands or tens of thousands patients treated with them. I think those are the right drugs to use, I don't think this is necessarily the best setting to use drugs that have had approval more recently or in which a smaller number of patients have been so treated, but the, you know, prophylactic treatment in the CRIM negative patients with drugs that have been -- for which there is an extensive track record of usage, seems to me the ideal setting in which to start. Obviously, just because, you know, thousands or sometimes tens of thousands of patients have been treated for a decade doesn't mean that there are no side-effects and that there's no risk to this, but it does give you a sense of at least what a risk benefit ratio might look like. And I agree anti-bodies over the long term, I

don't think will be good, and my guess would be, by perhaps extension to other diseases, that the longer therapies like enzyme replacement therapy are available the longer patients, and this is a good thing obviously, will be alive. And the more potential there will be to see the downsides of anti-body production over time, so, I would argue rather than wait for those things to happen, it would be good to start treating.

DR. DICKSON: You have a question in the back.

MALE SPEAKER: Yes, at least one speaker mentioned the potential of infections as being a -- something to look out for, in this world where, you know, potentially infantile and juvenile and adult on-site diseases are being treated with ERT, you have potentially complex medical cases where there are -- could be more than one condition, effecting the individual. Is this going to be a case by case basis where you're looking at whether, you know, off-setting the benefits and risk of therapy versus some kind of --

DR.ROSENBERG: [unintelligible] Can you repeat the questions [inaudible]?

DR. DICKSON: I'm sorry.

DR. ROSENBERG: [unintelligible]

MALE SPEAKER: So my question was, I guess, have -

- in the cases that have been put on ERT, are there side-effects such as infections which sort of or -- risk that outweigh the benefits of the therapy.

DR. DICKSON: Are there -- the question is; are there life-threatening, or very severe, infections that have occurred with the patients who have received immune-modulation or immune-suppressive therapy for enzyme replacement therapy, is that correct?

MALE SPEAKER: That's correct.

DR. ROSENBERG: So, I would certainly differ to Priya who has the most experience, but it seems to me the Pompe, infantile Pompe is the worst case scenario, because here you have kids who are -- whose muscles are very weak, they can't clear secretions, if anybody's going to get, you know, bronchitis and pneumonia and really a severe infection, I would think it would be these kids and yet they've done really remarkably well, so Priya, I would welcome your comments.

DR. KISHNANI: I would agree with what you say Amy. And there are situations when you've got to make clinical judgment calls, so although you have a protocol, it is in the clinical real-world setting, so if someone has a low white-cell count you can skip that dose of methotrexate and come back at it later, but the idea is it's -- you know,

you follow a certain group of drugs because you think they're acting by a certain mechanism of action and the key is to do it at the time of enzyme replacement therapy because, once you've escaped that period of time, the downstream of when to stop and how long to go is really challenging, and so in this real-world, I mean, honestly I'm one physician in the U.S. and these are children and babies from around the world that are doing it, and we're not seeing, you know, multitudes of deaths or anything from this. They're really sick kids and they are still handing this quite well. Often, you know, they skip a dose of methotrexate, or they post-pone a dose of rituximab, but it still has worked.

MALE SPEAKER: Alright [unintelligible], thanks my concern is just over the long-course, could there be a potential….

DR. DICKSON: The question is over the long-course will there be a potential risk? The question was over the long -course over the long-term any potential risk?

DR. BLUESTONE: So I think, you know, and it's probably worthwhile doing a full-literature review and doing it -- I spent a little bit of time looking at my computer while the question was asked earlier, and for rituxin plus methotrexate, and remember in most of those trials the

rituxin is being given continuously, or multi-dosing, which was not done here, there was very limited amount of adverse -- severe adverse events, either metabolic or infectious. As I mentioned before, there were a couple of cases of a disease-caused PML, there have been 14,000 human years of experience now with this drug, and there are a couple of cases of a virus infection so, I think that this drug -- and again it's for everybody to look it up, this drug is being, this combination of methotrexate and rituxin has been pretty well tested now, and has been, you know, considered, I think, a fairly safe therapy. It's not to say there aren't examples of infections, there are, but they are very limited and if you look on the label it'll show you exactly Hepatitis B, for people who are exposed is one that's come up as well. But I think overall the safety profile of this particular combination has been very good.

DR. DICKSON: Anne wants to make a quick comment.

DR. PARISER: Yeah, I wonder if this would be a good time to ask Angelo and Nickolai [spelled phonetically], if they wouldn't mind coming back to the microphone and continuing their previous comments because I think what was brought up -- because there's very limited experience in pediatrics and I wonder if you couldn't expand on that just a little bit.

MR. DE CLARO: Hi, sorry, Angelo de Claro with Oncology, my background is in adult hematology/oncology. So the -- [unintelligible], these two -- rituxin and [unintelligible], rituxin was approved in '97 and velcade [spelled phonetically] was approved in 2003, so certainly we've had more than a decade experience with both and the safety profile is correct rates for the indications for which they are approved for. So I think, really, we're going long-term safety, I mean, it's difficult to extrapolate, for example, the oncology population to this population, because of -- we are -- there are differences in the underlying disease, we're dealing with neo-plastic lymphocytes and neo-plastic plasma cells versus, these are not. And typically we give these drugs in combination with other cytotoxic chemotherapy drugs at higher doses that have been described. So, I think it's difficult to extrapolate the safety profile. Nevertheless, I think, mean, the effects of the adverse reactions being described, mainly infections, and mild suppression are consistent with what's been seen in the oncology literature.

DR. BLUESTONE: Isn't it true now, that rituxin has also been approved for ANCA-positive vasculitis?

MALE SPEAKER: That's correct.

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as well right?

MR. DE CLARO: So the experience with ANCA-positive vasculitis is a bit more limited. We have fewer patients exposed, our most -- most of our safety exposure database comes from patients with rheumatoid arthritis, which is the other approved indication, and it's in patients who are fairly sick, they have failed all the therapies including biologics, and in that patient population the safety profile was deemed acceptable, particularly with respect to serious infections. Rituximab is labeled, and you can go through label essentially, whatever's important and we know about it, it's in the label. Before I was referring to the pediatric -- the experience with rituximab exposure in pediatric patients, which is very limited at least, from the rheumatology side, and we were really concerned with more short-term toxicities not necessarily longer-term toxicity but also with the development of the immune system and whether giving rituximab to young patients might impact the development of the immune system, and whether they may develop [unintelligible] of long-lived plasma cells that they may need throughout life. And that's why in the patients with juvenile idiopathic arthritis, there are alternative treatments the risk benefit profile was not that favorable to require sponsors to these studies.

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DR. KISHNANI: Rekha, I'll try and address your question of the very young verses the older, I mean,

typically what we've done it in as young a two-week old, we've done it as young as in one-week old, and I think the oldest that we've done it, right now, is in a ten-month old in the [unintelligible] setting. And then of course, in the entrenched immune response it's been in older so, to me I think it's more challenging when you're doing it and, not just by the age, but even the stage of the disease. You know, if you've got a healthy infant verses a really sick infant, it doesn't matter if it's a ten-month old or a three-day old, I think it's more, in that situation, what I would be more worried. And then I think the other point is that, we have to really see, to me, the immune system is the immune system and so it's a therapeutic protein and you're -- you know, you're getting a response, so this class of agents, anything acting at the level of T-cells and B-cells to me, is what's important. It doesn't matter what that specific agent is, it's just that this has been tried, and there's been some success.

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upon the disease, the course of the disease, how severely effected somebody is, so at least you can in thinking about it, one variable is not a variable it's a constanus [spelled phonetically], the risk of a protocol. And then where the benefit will be, and where it will be seen or where a disease comes out in sort of a good-risk benefit ratio, will depend upon which ERT you're talking about. It also seems, just hearing this, and this is, you know, I'm newish to this area but, I agree with what Priya's saying, I think it's been a terrific demonstration of principal by her, Amy, Jeanine, and to a few others, that this can be done. So, it doesn't seem to me that, you know, the group today could or should necessarily decide this is the protocol, this is how we're going to do it. It's more a principal that it can be done, there are combinations of agents that work against plasma cells, B-Cells and T-cells that can be effected, and now it seems to me the decision is does -- do -- does the community have the desire to push this forward? If so, how do you get to consensus on which diseases, what regiment, and where do you start?

DR. DICKSON: Question?

FEMALE SPEAKER: Yeah, continuing on his point, so the regiment for naïve setting is actually just five weeks, right? If the patients are already identified as anti --

high anti-body titers, is the regiment longer? And so, there -- I think that's an important point, because if you start in the naïve setting, you're actually getting away with five weeks of dosing the long-term effects are actually not so long-term. Whereas if you go, you wait, you have high anti-body titers, they're actually exposing the patients to a lot more medications which might include bortezomib at this point, and a longer duration of therapy, and actually the risk-benefit ratio is actually tilting even to the worse, if you wait longer.

FEMALE SPEAKER: Is that correct?

MS. HOGAN: I think you are correct, and I would say too, that the toll of the up and down battle on patients, both health-wise and just looking at -- questioning the efficacy and trying to go back and get those under control later is such a vast difference from the prospect of doing a five week prophylactic treatment, that families who have now watched individual families go through this, you know, are horrified at the prospect of having to later come back and try to get under control high-sustaining anti-bodies.

FEMALE SPEAKER: And just to touch upon the previous topic where we were trying to see if different [unintelligible] can become paired against each other and

can we pick down to a titer as being some kind of a trigger for how treatment should be done, I am coming from the ousiande [spelled phonetically] of the world, and I've looked at a number of these assays and the titer can change depending on the dose that is being given to the patient -- within the same patient over time. So, without a clear understanding of what the assay is and what the titer really means, I would hate for anyone to pick something to a titer value and make decisions based on that, and I just think it's worth mentioning at this point that I think the titer should be a guide to see how one patient is performing over time, within that patient, but really beyond that it's not of very high value.

MALE SPEAKER: Chuck Willis Minneapolis [spelled phonetically]. I wanted to put a point on the considerations of different diseases and how this relates to some others. I think when you think about MPS-1 and MPS-2, remember that these diseases often present with multiple -- a history of multiple ear infections, sometimes pneumonias, so before they're diagnosed, they've already demonstrated they have a high risk for infections. So it is a different situation than Pompe disease. I guess the other thing I want to think about is for MPS-1 it's almost irrelevant when we talk about hernerstrum [spelled phonetically], every

child in the U.S., you're not going to say that [unintelligible], but I think that every patient to the patient goes through an ITA protocol that's called hematic rem [spelled phonetically] stem cell transplant. They get typically nowadays a couple doses of aldurazyme, then they go to complete myeloablation, and they get transplanted with a donor who's making normal enzymes. So, I think that's a specific and different, unique situation. So, I think there are situations where we definitely will have -- with better examples of when anti-bodies are impacting the effective drug and I think it still might be among those that have more atenuative [spelled phonetically] forms. We also have to think ahead to Sanfilippos syndrome, I think Charlie Richards will tell you published data; says that six out of 25 or more than 20 percent of patients with Sanfilippos syndrome has severe disorder basing, making no effective protein. So, there are some things to learn, it may not be exactly the examples we're thinking about and then finally, one question, this is an area of major ignorance for me, if we mandate an ITI protocol for patients with any severe disease or CRIM-negative disease, can we extrapolate from hemophilia? Does every hemophiliac, as soon as they're diagnosed, have an ITA protocol? Does that apply to factor nine as well as to factor eight, where factor eight disease,

they're unlikely to evolve anti-bodies. Can we learn from that experience?

DR. BLUESTONE: So, all great comments and great questions, I'd ask you a question, maybe you could…. So in my experience, bone-marrow transplant is not an innocuous treatment, and in fact is, makes patients, if anything, very susceptible to infections, especially in an aloe besides Grepher [spelled phonetically] host. So the community has made that an acceptable enzyme replacement therapy, for a patient population that has a lot of earaches before diagnosis. So I'm interested in your thinking about why in this case, actually potentially using tolerogenic therapies to make the enzyme replacement as robust as bone-marrow transplant is something that seems far more dangerous to you than a bone marrow transplant.

MALE SPEAKER: Well, I didn't say it was more dangerous, but I will say a transplant is more efficacious, and it's kind of unique to this set of diseases. Enzyme replacement therapy does not cross the woodring [spelled phonetically] barrier, even though I showed data to the opposite that was 20-fold normal therapeutic [unintelligible] doses. But by and large, kids with [unintelligible] will be aged -- will be dead by age 10, and during that interval, they'll have progressive neurologic

decline if they do not have a stem cell transplant. If you're going to Youtube, you can see one example, the only example I know of who's still alive, of a child who's had only enzyme replacement therapy, and you can see what the impact of that is. But for Martoler Mason [phonetic sp] MPS VI; for MPS I, and to a limited extent for MPS II, transplant will provide enzyme across -- or metabolic correction across the blood-brain barrier, prevent progressive mental retardation, and cervical cord compression, and paraplegia and quadriplegia. So I think the biology is unique to the situation.

DR. ROSENBERG: I would also like to add to that though that ERT is typically given before the transplant, and many of those patients generate high titer antibody responses. And although there's good success, there are a substantial number of patients who have graft rejection and graft failure, and that might be attenuated by tolerance to the enzyme, because here you're putting in, you know, allogeneic bone marrow, which in addition to the allo-HLAs, also has the peptides of the enzyme, and you have a prime T cell response ready to respond to that.

So I think that tolerance induction in that setting is still something to really consider as being potentially helpful. It might also put the patients in

better health to undergo the rigors of this kind of transplant.

MALE SPEAKER: Yeah, I think that's right. I think there's been more than 100 transplants done for Hunter's syndrome in the U.S., and I think there's probably six or seven now under experimental protocol who have had intravenous IV therapy before transplant, but so far as I know, that is largely [unintelligible] still.

DR. DICKSON: Thank you. So, I wanted to move the discussion back to idea of demonstration of clinical efficacy, because this is a major issue, and for Pompe disease, infantile Pompe disease, there's a fairly clear and grim end point. For example, in MPS I our canine research shows that the antibodies interfere with uptake into tissues, like the renal tubules, the joints, and the heart valves. Well, patients don't have clinically evident disease in the renal tubules, and they don't -- or they don't have renal failure, other clinical end points in kidney disease, and they don't -- and then disorders of the heart valve and joints may take decades to appear, and study. And so then the question becomes, you know, how -- and another example, in the canine study, the urinary GAG, which is a very clear biomarker, but not unnecessarily a meaningful biomarker in MPS I. urinary GAG is reduced with

enzyme replacement therapy even in the presence of antibodies by about 60 percent overall in patients treated empirically, and we found the same thing in the dogs.

If we tolerize them, we can improve that to say 86 percent, but in a rare disease with a lot of heterogeneity, how do you study even -- how do you make a clinical trial that will even detect that degree of difference, and that's -- and that's not even a meaningful end point. So any discussion on end points and efficacy, how we measure that?

DR. ROSENBERG: Yeah, I mean, I think it's certainly in terms of efficacy end points, it's very difficult, and you know, you point out the problems with UGAGs not being validated biomarkers, yet they are measures of the activity of the enzyme. I mean, I don't think anybody would argue with that, and as such, you know, when you start seeing increases in the -- or decreases in the percent reduction of the UGAGs, it -- that tells you something about the activity of your enzyme replacement therapy, and I think it's an important one, and just because it's not a validated biomarker for some -- for some hard efficacy end point doesn't mean it's not meaningful. I think it's very meaningful.

DR. KISHNANI: I would agree with Amy on this, is because we don't, say, have appropriate end points, or

they're hard to get, we still manage to do clinical trials, right? With all those challenges, and so now we're at the next level, where we're trying to see whether there's an impact of the therapy with and without the presence of antibody titers, but if we don't do this collectively, and start to move the field forward by gathering that data systematically, I think with numbers we will learn; we may not learn it from individual cases, and sometimes, it's pictures, it's videos that make a difference. You -- in a very, very sick Pompe baby, there is no real end point, you know, and as I said, if we hadn't really done careful -- so I can tell you that from those echocardiograms, I was told that it's stable; there's no problem. But when we went back and started doing the actual measurements of LVMI, we started seeing that there was a difference.

There is no [unintelligible] infant scale that you can use in really sick babies, and yes, the other ultimate end point is death. But what I'm saying is even in Pompe we did have our challenges as we were doing this, but as we collected this, and then we looked at it as group data, we started seeing the difference, Patty. So I think I would still urge drug companies in particular, you know, I often hear statements -- the enzyme, the antibodies make no difference, and so we're kind of done, and I want us to

think a little beyond and further out to -- before we make those conclusions.

DR. DICKSON: Other comments? Oh, Jessica Lee.

MS. LEE: Sorry, we didn't have enough room, so I'm sitting over here. I'm Jessica Lee, I'm one of the clinical team leaders in the Division of Gastroenterology, and [unintelligible] product. So I do agree with Amy that it's important, you know, shows activity -- the urinary GAG shows activity, but I think we can do better, and I think we should be looking for other measures that are more correlated with the clinical outcome, because even though that may show initial response, we have not been able to show a prolonged, I guess, effect on clinical end points, something that is meaningful to patients. And I understand the challenges, especially with some of the patients with, let's say, cognitive impaired patients; they're not going to be able to do a lot of these clinical measurements.

So I think, you know, we have a lot of smart people here, I think. You know, we should start thinking about what are some of the other maybe biomarkers or clinical end points that we could measure in a relatively shorter period of time, so that we can really know whether it's really working or not, you know? I think urinary GAG has served us, you know, well for a little white, but you

know, we could do better, and I just want to emphasize that point.

DR. DICKSON: Yeah, I mean, I agree, but also the problem is with -- at least with what our data show, are that those tissues, like the heart valve and the synovium, aren't necessarily -- the disease isn't necessarily reversible, because you get fibrotic change, and so you really aren't going to see an impact in the short-term. You're really only going to -- well, you may see an impact in the short-term, but I -- not a very large one. if anything, and all of also in those -- I'm sorry. One other thing is that we don't have yet, and perhaps we can develop these -- I hope we can, but we do not yet have terrific measures of improvement in joint range of -- in joint function, even though it seems like we should, we don't. And in -- also cardiovascular disease is difficult to assess as well, and so --

DR. ROSENBERG: But I want to point out what your data do show too is that you get much less uptake in the liver and the spleen, and in Fc receptor positive tissues, and so that tells you that, in fact, you're -- you stand a much better chance of getting that enzyme into the critical target tissues -- whatever those were; I don't know why if you were measuring those, you know, other tissues in the

dogs, why perhaps other tissues weren't sampled, but that data is very clear in showing that the antibody that -- antibody mis-targets the enzyme to tissues that don't need that amount of enzyme for clearance.

DR. DICKSON: Right, it -- the -- it's if you look at the clinical outcome measures that have been used for just assessing ERT and its efficacy, its, you know, stuff like, six-minute walk test, cardiac function score, pulmonary function tests, those sorts of things are probably not going to change substantially if you improve -- in a short period of time of getting better enzyme into the synovium heart valve and renal tubules, which has been the problem with the trial design. Now, in the long term, however, I fully -- I fully believe that we would absolutely make an impact on the disease if patients did not have the antibody titers, I do.

DR. UTZ: Patty, in the case I presented, we did see -- I don't think I mentioned this in my talk -- we did see a significant change in the number of infections a patient was having. So during that time when his neutralizing inhibiting antibodies were elevated, and his urine GAG was elevated, he was having chronic infections even before we knew that was the -- possibly the cause, the parents were complaining and saying, we don't know why he's

-- we just can't get him over this most recent cold, and after starting the immune tolerization therapy, even before his neutralizing -- or the percent inhibition was in a negligible or insignificant range, the infection stopped. So what that the IVIG or was it really having the enzyme more accessible? We don't really know, but because we were giving the two doses, you could theorize about some pharmacology mechanisms, possibly that first dose in the morning, if the antibodies were binding to it, maybe they were hanging on to it for a while; we give the second dose later, maybe that was more effectively used, because the antibodies were busy with the first dose; there is some, you know, we've tried using that kind of a process to manage other types of infusion reactions, but for some reason, the infections stopped, and they have remained under control, so that's one thing we could monitor. I don't know if there's a good systematic way of monitoring that, but --

DR. DICKSON: Thank you. So maybe these n of one studies will teach us something, and help us find a path forward here, which leads me to the next question I have on my list, which is reimbursement. So I don't know if Jeanine or Priya, you want to talk about how one gets reimbursement for these sorts of [inaudible].

DR. ROSENBERG: Before we get to that, can I just

ask one question? So one thing that popped out at me during the course of this meeting is how little we understand about these kinds -- the hypersensitivity responses that seem to be so pervasive that nobody's looking at the mechanism by which they're generated, whether they're IGE-mediated, IGG-mediated, and that these kids are treated with, you know, heavy doses of steroids and antihistamines, which are not benign, and in fact, you know, may have an effect on susceptibility to infection; we know steroids are good at that. And as well as just keeping a kid very drowsy for a long period of time, and perhaps if we better understood the mechanisms by which those kinds of hypersensitivity responses are mediated, we could use some of our more -- our newer armamentarium to address them.

So the anti-IGE, I think, is a great example. I think, you know, if these are IGE-mediated, then taking, you know, perhaps taking Omalizumab for -- you know, once every few months or whatever the -- it would call for, would be more benign, certainly, than having to have -- be chronically dosed with steroids and antihistamines. So something to consider for further evaluation, and perhaps consideration of therapeutics.

DR. KISHNANI: Amy, I would love to comment on that as well. For our Pompe patients, we did not routinely

pre-treat. I know for certain ERTs, I was actually surprised to hear that there is a pre-treatment regimen that's done. We do not do that, specifically for the reasons that you mentioned, is you don't want steroids; it's -- it can induce a myopathy. I know it's just once every week or once every two weeks. And we do -- if we do see a reaction, we do check compliment, tryptase, and IGE. If they're IGE-positive, I do not like to pre-treat with Benadryl, because you're only masking what might become a full-blown reaction. So in that situation, we try and manage without it until there is a reaction so you can do it with, you know, a desensitization, or starting at a slower rate.

And we've had some very good success, again, with Pompe, and we've published on this as well. And they can go back without any kind of pre-treatment by, you know, slowing the infusion rate, and doing it in smaller doses, more often, et cetera. So I don't know -- I know that for certain ERTs it's almost like -- along with the ERT comes the pre-treatment, but we do not do that.

DR. DICKSON: Any comment on reimbursement and how it gets --

DR. KISHNANI: I can comment on it, at least for our -- for what we do for Pompe. We're not having any

problems right now, because we've published on it, it's almost like a standard of care. We hear this from around the world that patients are getting it, we're not really having a challenge with it where reimbursement is possible. And in certain parts of the world, like in Latin America, where IVIG or rituximab is expensive, you know, they're unable to do it, because this is private insurance trying to cover for it; or in places like India, but here in the United States, we've really not had a problem with reimbursement for it.

DR. TANPAIBOON: [unintelligible] Priya is trying to teach me how to do immune modulation on the patients they have in D.C. I was lucky to have immunologist, and he's oncologist as well, so to help me to go through that process, because at beginning when we tried to do immune modulation by using Priya's protocol, insurance denied that. So we have to do as inpatient for three days, because of the, you know, that's how to get it done, but after that, we could do as outpatient, and we could go through everything. I did not deal with insurance, but I -- my colleague did and we could [unintelligible].

DR. DICKSON: Jeanine, do you have any comment?

DR. UTZ: Well, I -- the second -- next to Priya, I'm sorry. What country were you -- was that in the United

States?

DR. TANPAIBOON: No, we're in D.C. We're in D.C., here in D.C.

DR. UTZ: Oh, okay, okay. So I think in most cases you will be able to get insurance coverage, and it is because of what Priya said, if there's any post marketing or studies published on this, which Priya has done, it supports the use of an immune tolerance regimen, so most insurance companies will cover these expensive treatments if there is published literature supporting it, and they are looking for at least two pieces of literature. I don't know with the healthcare reform if that will -- if we'll see changes in that. In our patient, we actually wanted to do a higher elaprase dose, and the insurance would not cover a higher dose. And you know, looking back, it's probably just fine. So we did -- they would cover up to one milligram per kilogram, and I don't know why they made that distinction there. So we did increase the dose from the FDA-approved dose of 0.58 up to one milligram per kilogram. I know they were looking at some things in the package insert for that. But I think you can get coverage if you can support it with published literature, so…

DR. TANPAIBOON: Yeah, I could get the coverage, because at that time, we had a publication from Priya, and

for the enzyme replacement therapy in general, when we tried to increase the dose, because you know, we think that might be helpful to patients, and the insurance denied that, because they said there was no publication to see the -- to provide the efficacy whether or not that high dose does improve the outcome the treatment, but because of the personal experience that we use high dose of enzyme replacement therapy before and we saw the improvement, but we couldn't use that personal experience as the acumen with the insurance company.

DR. HOLLAND: And I think it's important to note though that the publications are on Pompe, and getting insurance reimbursement for some of the other LSDs is and has been problematic. Specifically in Hunter syndrome, I know -- I've at least one family that has been flat out denied by their insurance company for any immune tolerance drugs or regimen. And so I think, like I said, it would behoove us to have some kind of results or white paper that would assist families in that endeavor.

DR. UTZ: You know, I agree with you. So with Priya's situation, there's plenty of literature supporting it; with our situation, I was not doing rituximab, I was not -- they would have covered Methotrexate, because it's inexpensive, so that -- they don't care if it's inexpensive;

they don't care what you give the child if it's inexpensive. But there may have been issues with the rituximab, but we chose not to do that. The IVIG, the insurance companies in most cases will cover that without question, if you have some sort of clinical rationale for it. So I don't know if there's a general rule of thumb. A lot of these things are kind of -- the number of years and history of use of these products in other conditions play into it too, but I do know that you're -- you kind of have a sure thing if you have something published on it.

DR. DICKSON: Any -- and does anyone else in the audience have any experience with this that they'd like to share? Well, I have one question. So you're able to get reimbursement for the treatment, but I assume you had to collect a relatively large amount of data, and have someone handle that data, and store the samples, and all of that. So how -- do you have any idea how much unreimbursed expenses and time these things cost?

DR. KISHNANI: If I'm understanding your question, you're asking for collection of this data?

DR. DICKSON: Yeah, I mean, if you're going to use these as a way to better understand the use of the immune tolerance protocol, I assume you're collecting data.

DR. KISHNANI: Right, this has been the labor of love, and sweat, and tears, through years. A lot of it has not been covered; it has been my personal time, but I think over time, we have had some funding now from the LDN, and also from Genzyme. But a lot of it has been what you call unfunded research.

DR. UTZ: I think in my case, too, I put a lot of extra personal time into this to try to work through the details. The lab processing for the neutralizing antibodies, right now, Shire was, you know, doing that complimentary, as they do for all their patients. They're moving their lab process to LabCorps, and it will -- I believe it will still be under the Shire umbrella of something that is not charged to the patient, but I think processing those neutralizing antibody labs isn't -- is an expensive lab to process, relatively speaking. So there may be a time when we're ordering these under clinical care, at least these labs, or doing this under clinical care; we're sending these labs in to Shire, but they're being treated as sort of research labs, even though it's really clinical care. So if we start to bill the lab processing to clinical care, I don't know if that will become an issue in terms of cost coverage.

DR. DICKSON: And you're talking about the

antibody response --

DR. UTZ: Antibodies, yeah. And I guess that diverted a bit from your actual questions, but --

DR. DICKSON: No, no, that's good. That was next on my list.

DR. KISHNANI: If I could make one plea, Anne, just for a moment, as I know many members from industry, you know, there are registries, and there is data collection in these registries, and as part of this learning, if there was some more detail put into this kind of follow up, or doing sub-registries with special focus and attention, because it's a lot of detail, and you're not going to get it, you know, from patients who don't need it in a general registry. But my plea would be, you know, some approach to this, and using some common data elements to try and understand this in the field would be terrific, and we would learn a lot from it.

DR. DICKSON: Yeah, Anne?

DR. ABICHANDANI: Can I ask a question? Sorry. Priya, are you proposing a clinical trial to study this, or in the context studying immune tolerance regimens in the context of registries? As you mentioned, you know, most of us have registries for different diseases.

DR. KISHNANI: Yeah, in the context of a registry,

I mean, how do you capture clinical information otherwise? I know I have lots of it, but not had time to input it, but if, you know, there was some support for it, there would be a lot of good data generation that would occur, and so if as part of registries, if this becomes a component, I think would be extremely helpful.

DR. DICKSON: Anne, and then Joe.

DR. PARISER: Yeah, I think that brings up a couple of things that have been touched on a little bit earlier as well. That's that there is a fair amount of information now; we do have several decades of enzyme replacement experience now, although, you know, only in a few diseases, but we also have a lot of work now that you and others have been doing, so you know, how do we get this out then more to educate the community, because what you're describing is a lot of your personal time of individuals, but do we know enough now to put this into more general recommendations? But also in terms of study, I'll bring it up again -- I brought it up earlier -- got a little controversial, but are we at the point now that should be studying more pre-market? And I was talking to the review division in the office at the break, and they said that they are very open to discussing this with the commercial manufacturers for how do we incorporate these into

protocols? Can we do a better job in labeling as we get more of this information, because will this help mitigate some of these problems that you're describing?

DR. DICKSON: Joe?

MALE SPEAKER: I think in terms of the -- if you look at the need in the community, if you take MPS II for example, there is no public data. I think it's very difficult to do these trials for individual physicians. You almost need -- Priya had a unique advantage early on in the trial where she had lots of patients coming to her site, and had outside support, but for -- now we don't see that number of patients coming any one site. To do an MPS II immune modulation, you almost need to have a clinical trial done in one or two sites, and support it by whatever revenues you can support that to get it really that initial data, say should we continue on and do a lot more?

DR. KISHNANI: Joe, I wish what you said was true. The patients were not coming to my site, and there was no support. I just did it because the babies were dying, and so I started one at a time, and it was -- it was a long road, and probably could have been done more efficiently if it had been done in a more systematic way, as you suggest.

MALE SPEAKER: But the nature of the Pompe patients are so different compared to the MPS patients,

where you don't see the acuity of disease, which makes it a little different driving force, because it's not the same driving force in terms of creating the severe MPS II patients, for that matter, or some of the MPS VI patients who have severe, you know, mutations that give you antibody response.

DR. DICKSON: Jeanine, you have a questions?

DR. UTZ: I have a cab waiting, so I just [inaudible]. Okay, thank you.

DR. DICKSON: Barabara?

DR. BURTON: With regard to the MPS II patients, though, I think that the severe MPS II patients who have the complete gene deletions, or rearrangements, I mean, they are the obvious tip of the iceberg that Priya alluded to earlier; they're the so obvious easy patients, where now we clearly know enough that those are the patients where, with absolute certainty, they should now have the immune prophylaxis when they present. I mean, I don't think there would be any excuse ever to let a patient like that start their therapy without immune prophylaxis, because I think you know, these immunologists have convinced me certainly, and what Priya has said has convinced me that this five-week course is very safe, and we know for sure that those patients are going to develop high titer antibodies -- of

course they will. And now I'm also hearing, and it's giving me tremendous pause from these guys, that even the patients who don't have obvious adverse effects, having these antibodies for whatever reason, and in whatever titer for years and years is probably not good for you. I'm kind of wondering about all the patients, but that subset for sure. I mean, it seems like, you know, if we're going to say anything, we say that those patients need to be treated from the outset.

DR. HOGAN: I think that brings up something that I don't want to gloss over, and that is, at least in our population, the time to identify genotyping and CRIM status, and so when your child is diagnosed and they say, "And there's a treatment" -- I actually dealt with a family who said, well, you know, we understand this whole antibody thing, and we could wait to find out if he has a severe genotype, but I want to do something, and they didn't wait, even knowing this latest information. So I think the turnaround for some of these families is one, two, three months, and so I think that's problematic, and needs to be changed.

DR. BURTON: Well, that's another place where we also need physician education, because I think some of the physicians aren't even getting, you know, the molecular

testing early enough in the course of the diagnostic evaluation, or taking a little bit leisurely approach to that too, so that needs to be incorporated.

DR. KISHNANI: Melissa, I would agree with you, and it used to be the same, even for Pompe, when, you know, it was first dependent on western blot, and it took time, and then I had to push our lab. You know, we are doing it now with a two-day turnaround, so by the time the enzyme is at that facility, the mutation results are back and it can be done. So I think that can be -- that is a doable situation; it's just the question of readiness that is this the sub-population, or is this the group, or what is the group that we want to look at?

FEMALE SPEAKER: Quick question. Do the mutations actually -- can you say this -- a certain mutation is higher likelihood of being antigenic?

DR. KISHNANI: Yes, absolutely, for --

FEMALE SPEAKER: Yes, okay. That's the --

DR. KISHNANI: -- you have two deleterious mutations, and we've put a mutation database now on the website, on our website for anyone across -- in the world to see for Pompe. And I think for something like an X-linked condition, where you've got a gene deletion or rearrangement, it should be very simple and just a question

of collection over time, you know, to convince ourselves, or you know, which ones are the ones. Even if one went back retrospectively and looked at that, and seen which of the patients who had the high titers, I'm sure you'd come up with a list of genotypes.

MS. RICHARDS: Again, Sue Richards from Genzyme. I'd like to add to that last question. So we have had in the past two post-marketing commitments to try to look for what were predictive factors that could cause an immunologic response in patients. And it was a pretty exhaustive evaluation, which we looked at genotyping, we looked at HLA, we looked at CRIM status by western blot, we looked at enzyme activity, and we also looked at enzyme protein by [unintelligible] as another assessment for CRIM status. So with respect to the null patients -- so the ones that are CRIM-negative -- and with a few other exceptions that it was very clear they amounted a high antibody response. Beyond that sub-population, it got very murky. It was really -- you had exceptions all over the place. It was very difficult to say that a particular genotype that you had once you got into splice mutations and so on, basically could correlate in terms of predicting the kind of antibody titer that you had.

So just wanted to put it out there, because we're

basically giving patients a normal enzyme, but the genotype can lead to various abnormalities, of which some of them are conformational, and so it ends up being very difficult to make those associations. At least, that's what we've had -- our experience has been in the past.

DR. KISHNANI: Sue, but you would agree that the ones who are CRIM negative, you can identify them by genotype?

MS. RICHARDS: Oh absolutely.

DR. KISHNANI: And I think that's --

MS. RICHARDS: Yep.

DR. KISHNANI: That's the first starting place, I think, for many of these other conditions. You know, I think our lessons from Pompe have taught us a ton about that.

MS. RICHARDS: Right, so -- I mean, CRIM negative, from the genotype you could predict it was going to be a CRIM-negative patient, but beyond those and a few that were very large truncations, and so on, then it got very difficult to go from genotyping and to be predictive of what the antibody response was going to be.

DR. BLUESTONE: Can I make a comment? On my way out the door. I think that's a really interesting point, and something that this group and others should think about,

and that is that -- we were discussing this first thing this morning. As an immunologist, I -- if I had to design the perfect setting to give a protein, it's a setting like this, in which we know from a Nobel Prize winner, Sir Peter Medawar, that neonatal tolerance is the most common way to induce tolerance. Secondly, that giving a protein IV without any adjuvant is about the non-immunogenic, and yet, in spite of that, many of these individuals get the -- so it's telling us something about the status of the individual at the time that they're getting this protein, and I think that the discovery effort there could be a really important thing, because it may not be something about the H allay and the prone to the response; it's why certain individuals are protected against it, and what it is in there that's the regulation, or something that's doing it.

So I think your point's well taken. Until we really can figure out what the parameters are that determine response or non-response, it's going to be hard. Now, my default position is treat everybody, as I've already said, because don't worry about it, but if one's going to be very sensitive about that, and certain of the diseases, I think doing some more studies -- and this is something the NIH should be interested in funding, because it tells us something not just about the response to this protein, but

about the nature of the immune response early in someone's life, which may impact everything from allergy and asthma, and peanut allergy and all of that, so I would encourage this group to sort of lay down that this is something that needs to be understood better than the nature, shape of the immune system at this early age, because that's going to tell us a lot.

DR. DICKSON: Rekha?

DR. ABICHANDANI: So I had one additional question for the panel. I think this was alluded to a little bit earlier. How does one weight the risk-benefit of an immune tolerance regimen for, say, an intrathecal therapy when we have an [unintelligible] twice that we're investigating? It's particularly if you're thinking about doing this pre-approval, you know, that's an added infection risk now, just something to think about, and if there were any thoughts from the panel, I'd be very interested.

DR. DICKSON: I mean, so far the majority of the patients have been treated with intrathecal enzyme replacement therapy, and Hunter and Hurler-Scheie studies have been non-naïve; however, there's appreciably new therapies being studied now that are not. So yeah, I mean, we don't -- we don't know, right, the impact on the antibodies. We do know that there's in -- in our canine

models, we see a plasmacytic infultrate and meninges, and they -- there is a possibility of that -- of antibody response in CSF to the enzyme.

Okay, I have one last -- one question, which is the fact that we alluded to that there's not a commercial assays available for antibodies, and I wanted to kind of throw out what people think the barriers are to that. Well, if a lab were to -- maybe, Rekha, you can answer. If a lab were to try to develop an assay for antibodies against one of the proteins that Shire makes, would Shire provide that protein to that lab to develop said assay?

DR. ABICHANDANI: I think Sue and Yong Chang [spelled phonetically] can probably also talk about this. I mean, we haven't developed these assays thinking of a companion diagnostic. I mean, we have -- I think most companies and I think Gary [spelled phonetically] is also there in the audience. I think companies have typically provided this as a service to physicians.

DR. ROSENBERG: So I can't -- why can't physicians send in samples and ask for antibody testing?

MS. RICHARDS: So again, Sue Richards. I can only talk about experience at Genzyme across our replacement enzymes, but we do run antibody testing, as I said earlier, for serum samples from patients that any treating physician

can submit. These are run at no charge, so they're considered part of the service, and the treatment regimen in terms of that comes with the drug. So we do it, as I said, for binding antibodies, for the two neutralizing antibodies; if there's a concern about hypersensitivity reactions, we do drug-specific IGE; there are other mechanisms also by which one can have hypersensitivity, so we also have available that we can run a serum tryptase is another marker. We also do compliment activation, because you can get mass cell degranulation; mass cells are the cells that'll give you your -- they de-granulate and they have pharmacological mediators that cause your allergic-type symptoms, and it can be also done via compliment activation.

So it doesn't have to be through an IGE mechanism. So we do study the biology behind mechanistically what's happening, and try to liaison and worked together collaboratively with the physician. We do in Genzyme in a CLIA laboratory. We can inspect it every two years, because we're submitting patient information that's used for treatment purposes. And it takes a while to maintain the length of these assays in a validated state over years, so as we change reagent lots, we go through an extensive crossover of reagents. So there's ways to do this. Other companies do outsource, but these antibodies and services

can be provided, so it is a possible approach.

DR. DICKSON: Thank you.

MS. RICHARDS: And I have colleagues here who can talk about their approaches.

MALE SPEAKER: This is Gary [unintelligible]. We do the same thing, and we'll provide for Naglazyme, we have a commercial lab, that outside lab that does our work. It's a CLIA lab, because they're -- it's going to use it for reporting to the physicians. We're also gearing up with Vimizin [spelled phonetically] to have our IGE assay, our total antibody assay, and our neutralizing assay available for the -- that product also, and that's where the standard that we set for all of these. So it is available.

DR. DICKSON: Great, thank you.

MALE SPEAKER: [unintelligible] also applies to Shire as well. And actually, in addition to that, we also provide free testings for [unintelligible] GB3s, the biomarkers, as well as [unintelligible] and others. And so it -- but Priya, you know, prior to several years ago, we always run it in-house, but now we're in transition to -- and also, it's all these assays to [unintelligible] in order to increase the turnaround time.

DR. DICKSON: Thank you.

MALE SPEAKER: I guess the question also is around

turnaround time. Is there -- I don't know, are there any physicians here, like that speak to any of the turnaround time they've seen with maybe Naglazyme in any case, or any of the other products?

DR. DICKSON: I don't know.

MALE SPEAKER: Sounds like there's been a long turnaround time as an experience, so maybe that's something I need to look into. [laughs]

DR. DICKSON: I don't know. Barbara, do you have any experience with that? I don't know. I don't have any experience with that.

DR. BURTON: Oh yeah, I mean, I think the turnaround times are really long with all of them, really. I mean, as compared to what we're accustomed to with commercial laboratory testing, I think -- yeah, I don't remember specifically what it's been lately with Naglazyme as compared to, you know, the other enzyme products, but I think they're really long. Yeah, we're talking months.

MALE SPEAKER: Then if you were to order, how often would you want it -- intervals would you look at testing?

DR. BURTON: Well, I mean, I -- I'm not sure what the right interval is. I don't know what people think. I send them every six months, so I like -- you know, I

typically get them on all of my patients on ERT every six months. You know, I don't know if that's the right interval; maybe we should be getting them every three months; maybe it needs to be every three months in the beginning, and then it could be every six months if they're, you know, not going anywhere. I'm not sure how it should be, but I do it typically every six months, but you know, sometimes it takes six months before we get a result back. So yeah, it would be nice if we got them, you know, a little bit faster.

DR. DICKSON: Yeah.

FEMALE SPEAKER: I also wanted to offer a perspective from immunogenicity testing area. I'm with Alexion, and we do arrange for rapid turnaround testing of anti-drug antibody for our Soliris product on the market. And we do provide a pretty rapid turnaround. One thing I wanted to bring up though is that with some of these commercial laboratories, these very specialized and molecule-unique tests, they actually fall into their esoteric testing category. That means they generally charge a lot more, and for example, LabCorps, it falls into their esoteric testing. Also, if there's a very long lag time between samples, sometimes assays need to be revalidated at certain intervals every six months or 12 months, or at least

re-qualified with their reagents. So I would invite physicians to collaborate with the companies to talk about these time frames, and when samples might be expected, so that -- so you can arrange for that kind of rapid turnaround when you need it, and it involved a lot of communication about what's necessary, but if a lab is receiving a couple of samples every six months, there may be quite a bit of setup involved to be able to do that test.

DR. DICKSON: Thank you.

1:10:00 onward

-- thank you. Any other comments? Okay, thank you. I'm going to turn it over now to Jessica Lee for some closing comments.

Closing Comments

DR. LEE: Thank you. So, we've had a really productive day today. I know many of you have traveled very far and need to get back, so I'm going to try to make it as brief as possible. I'm Jessica Lee; I'm a Medical Team Leader in the Division of Gastroenterology and Inborn Errors Products. So I'm going to briefly summarize kind of what we've talked about today.

Today we discussed the impact of anti-drug antibodies on ERT, and the role of implement immune tolerance induction to reduce potential clinical impact of antibody development. We began our first session with Dr. Amy Rosenberg's nice overview on the immune response to ERT and FDA experience. Then we heard from Dr. Barbara Burton, a highly-regarded clinical expert, who provided a clinician's perspective on the impact of ADA in patients with lysosomal storage diseases. Then we heard from Dr. Rekha Abichandani regarding how industry generally approaches immunogenicity information and assay development during drug development. Subsequent to these very informative presentations, we had a lively discussion on the impact of ADAs on clinical outcomes in patients with LSD. The panel members acknowledged the difficulties in assessing the impacts of ADAs when there aren't commercially available

assays to measure antibody information, which is further complicated by difficulties in measuring clinical outcome in certain patient populations where the disease presentation and progression are heterogeneous.

But we did hear some of the good perspectives that if we were to get -- if clinicians were to be able to be provided some historical data on assays at the time of assay reports, that that would be helpful for them. In addition, we could -- there could be some improvements in the turnaround time of these assay results. Most importantly, we learned that the long-term data on immunogenicity is very, very important because it often takes a long time to see the effect of antibodies. And immunologists actually pointed out that we need to do a better job in understanding what is immunogenic about the protein so we can better target the assay development, and also better understand the effect of antibodies. So just because screening antibodies says it's positive and that there's no correlation to clinical outcome, it doesn't mean that the work is done.

We then launched on to our second session, where we discussed the rule of immune tolerance induction in ERT. We started off with Dr. Larry Turka, who provided a nice overview of what we mean by immune tolerance induction and various pharmacologic approaches to immune tolerance, and he

shared some of his experience in transplant immunology. Then we heard from Dr. Priya Kishnani, who's a world-leading expert in the immune tolerance induction in infantile-onset Pompe disease. She has shared some immune tolerance induction algorithm that seems to be working very well and could negate intantile-onset Pompe patients, as well as high-sustain antibody titer patients. The results were quite compelling. The question remains, can these regiments be translated to all other LSDs? And we've had a lively discussion on that.

Then we heard about -- from Dr. Jeanine Utz on a case study of her patient, who's been receiving kind of a different approach for her Hunters's disease patient, with an increased ERT dose intensity along with IVIG. She shared that this approach may be appropriate as an initial step in some of the patients where they may be at high risk for developing infections and where it's acceptable to achieve immune tolerance over a longer period of time.

And lastly we heard from two patient representatives, and we really thank Mr. Steve Holland for filling in at the last minute, but we really enjoyed hearing your stories and thank you so much for sharing your stories on Spencer, Maddie, and Laney. That was really helpful and it almost brought tears to my eyes. Then we heard from

Melissa, and thank you for sharing your friend's story. We're sorry that we couldn't meet Jamie Fowler in person, but the story is very compelling and it was informative for us to hear her experience with her son.

The panel then discussed multiple important topics, some of which include the target population who should be treated with immune tolerance induction, and some actually stated that maybe prophylactic immune tolerance should be considered in negative patients, although this is not an advisory committee meeting and we're not here to really make decisions, but it was really helpful to hear from everyone what their thoughts are. We also heard about some of the methods of assisting efficacy in immune tolerance, which is always a challenging issue. We discuss that internally a lot, and it was nice to hear from everyone else what their thoughts are and to what degree can we translate what we learned from our experience from one disease group to another group, and that was very good for us to hear.

It's clear we have a learned a lot over the years, but we also have a lot to do. I'm glad that all of us had a chance to come together today, because this is the first step to working together to make sure that we make a difference. We met to identify knowledge gaps and discuss

some of the future directions, and I'm an optimist and I really think that we've made a really huge step today. The majority of our patients have only one ERT available to treat their condition at this time, and so it is our common goal to optimize their therapy by identifying any barriers to successful treatment and finding best solutions to address them.

So I'd like to conclude by thanking those who've made this workshop possible. First of all, we could not have done this without enthusiastic support from NORD and their co-sponsorship. Particularly, I would like to thank Ms. Allie Freitas and Mr. Derek Gavin for their tireless effort throughout the planning process as well as today, manning those tables and you know, making sure that everything's running smoothly. I would also like to thank BIO for nominating our industry representative to serve on the Steering Committee. I really do think that we all need to work together, so it was really helpful to have Dr. Abichandani represent industry during out Steering Committee meetings. I would also like to thank the Steering Committee members, consisting of the NORD representative as well as their nominees from Children's National Medical Center; industry representative nominated by BIO; academic, clinicians, and researchers who were sitting on the panel

today; Ms. Melissa Hogan, who served as a patient representative throughout the planning process; as well as many FDA colleagues from both within the division and outside the division, particularly Dr. Amy Rosenberg and Susan Kirshner from the Division of Therapeutic Proteins, Dr. Emmanuel Elicana from Office of Biotechnology Products, Dr. Anne Pariser from Rare Disease Program, Drs. Yow-Ming Wang and Christine Hwang [spelled phonetically] from the Office of Pharmacology, Dr. Lynn Yow [spelled phonetically] from PMHS, Dr. Angelo DeCarlo from Division of Hematology Products, and Dr. Nikolai Nikolov [spelled phonetically] from the Division of Pulmonary, Allergy, and Rheumatology Products.

This was certainly a large group effort that would not have been possible without everyone's contribution. I would like to say special thanks to our moderators, Drs. Anne Pariser, Patty Dickson, and Priya Kishani for facilitating the discussions today and running today's workshops so smoothly. Lastly but importantly, I would like to thank my colleagues, Dr. Laurie Muldowney, a star Medical Officer in our division, and Ms. Moreen Dewey [spelled phonetically], another star, our Senior Regulatory Project Manager from our division, who served as my co-lead during the planning process, and I couldn't have done this without

them. On behalf of our division leadership, Drs. Donna Griebel, Andrew Mulberg [spelled phonetically], Joey Corvic [spelled phonetically], and our office leadership, Dr. Julie Bites [spelled phonetically] and Dr. Amy Egan [spelled phonetically] and all of my colleagues at FDA, I would like to thank all of you for participating in this important workshop today, and for contributing to the productive discussion. And special thanks to patients and the parents for joining us today and sharing their stories. We look forward to continued collaboration, and I hope that you have a safe trip back home. Thank you.

[applause]

[end of transcript]