Part I: Pompe Disease from Diagnosis to Treatment

Operator:

Welcome, and thank you for standing by. At this time, all participants are in a listen-only mode. At the end of this conference, there will be a question and answer session. If you would like to ask a question at that time, please press *1. Also, I would like to remind all participants that today's conference is being recorded. If you have any objections, please disconnect at this time. I would now like to introduce our moderator for today's conference, Mr. Patrick Hopkins. Mr. Hopkins, you may begin the call.

Patrick Hopkins:

Thank you, this is Patrick. I'm chair of the APHL Newborn Screening Quality Assurance/Quality Control subcommittee of the Newborn Screening and Genetics in Public Health, and the Newborn Screening Laboratory Manager in the state of Missouri. On behalf of the QA/QC subcommittee, I would like to welcome everyone to today's webinar, the first of a two part series entitled, "Pompe Disease 101: Clinical Aspects and Screening Methods."

Today, we'll be having three excellent presentations from experts in the field. The first presentation will be an overview of Pompe Disease and clinical manifestations by Dr. Alex Kemper. Dr. Kemper is a professor of pediatrics at Duke University. He's a general pediatrician and health services researcher. Dr. Kemper is the chair of the Condition Review Workgroup for the Secretary's Discretionary Advisory Committee on Heritable Disorders in Newborns and Children. In addition, he is a member of the US Preventive Services Task Force, and Deputy Editor of ***Pediatrics***, the leading journal of child health care, and the official journal of the AAP.

The second presenter will be Dr. Olaf Bodamer discussing diagnostic issues. Dr. Olaf Bodamer is the Chief of Clinical Genetics and Medical Director of the genetics laboratories at the University of Miami.

The third presentation will be by Dr. Neena Champaigne, who will be discussing treatment of Pompe. Dr. Neena Champaigne is a medical biochemical geneticist and Director of the Metabolic Treatment Program at the Greenwood Genetics Center in South Carolina.

Please save all questions and comments until all speakers have presented, and the operator will give instructions on how to submit questions verbally and electronically at that time. At that time, Patricia Hunt, a member of the APHL QA/QC Subcommittee and manager of the Texas Newborn Screening Metabolic Group will oversee the question and answer session.

And now for our first speaker, Dr. Alex Kemper.

Dr. Alex Kemper:

Thank you very much, Patrick. (inaudible) I'd like to be able to talk about Pompe disease, and I would also like to thank APHL for putting together this excellent two-webinar series on Pompe disease.

I'm going to ask that you all move my slides for me; I'm beginning right now on the first slide. My title slide, "An Overview of Pompe Disease and Clinical Manifestation."

And now moving to my second slide, "Pompe Disease." I think, as many of you know, Pompe disease is caused by deficiency of a specific enzyme, acid α-glucosidase, or GAA, and that leads to accumulation of lysosomal glycogen. It's an autosomal disorder, and there are many, many mutations that have been described, now

numbering more than 300 of them. And one of the challenges with Pompe disease is it really has a broad spectrum of illness.

So, there are many different ways you could classify Pompe disease because of that broad spectrum of illness. I'm presenting here one of the common ways to think about it. It's typically broken up into the infantile form, which is the most severe form, and really the target of newborn screening, and the late-onset Pompe disease.

So as I mentioned, the infantile is the most severe from, and typically has onset within the first year of life. And it can be further subdivided into two types. There's the "classic form," which is an infantile onset with cardiomyopathy. It's associated with progressive hypotonia and cardiomyopathy as I mentioned, and without treatment death is usually within the first year of life. And then there's the infantile onset form without cardiomyopathy, or the so-called "non-classic form." Again, as you might surmise with what I just said, it's not associated with cardiomyopathy. These infants do tend to live longer, but without treatment they also die in early childhood.

Now, contrast that with the late-onset form. That has quite a variable presentation. Again, it would present certainly over

one year of age. So what's interesting is that most individuals with the late-onset form develop symptoms in adulthood, but the diagnosis usually takes about eight to ten years, and then death some 27 years later, so certainly a more indolent form that can be difficult to diagnose. Individuals maybe deal with mild weakness in childhood that can go unrecognized, and then develop a slowly progressive myopathy. It's variable in terms of the long-term outcomes without treatment. Some individuals become wheelchair-dependent, or require mechanical ventilation, and ultimately come to respiratory failure.

In early infancy, it can be difficult through -- certainly newborn screening, to determine first, who has the infantile form versus the late-onset for, if the individual doesn't have cardiomyopathy. However, mutation analysis can help with that significantly, and I believe Dr. Bodamer will talk more about that in his presentation.

The treatment is enzyme replacement therapy. Basically, you're providing back the missing enzyme. There are two forms of the enzyme replacement therapy. There's the form that was FDA-approved in 2006 that is really indicated for the infantile-onset form. And then in 2010, another product, Lumizyme, that

was FDA-approved. And that's approved for individuals that are eight years old and over.

The key thing to take away from this slide is that enzyme replacement therapy is not curative. So, affected individuals will need to continue to have infusions over their life. It's typically given every two weeks due to a central catheter, and adverse effects associated with it include mild infusion-associated reactions, although some individuals can also develop antibodies, and if the antibody titer that we develop is sufficiently high, it can inactivate the enzyme, and I'm going to discuss that in greater detail in just a couple of minutes.

So, there are some factors that can affect detection that those of you who work in the laboratory should be aware of. First of all, screening could potentially identify individuals who are carriers. They can have below-normal enzyme activity levels, and therefore be picked out that way.

Perhaps more challenging, though, is the issue of pseudodeficiency. So individuals with pseudodeficiency have low measured enzyme activity levels, but they actually have functioning enzymes, so although it will look like they have Pompe disease, if you only look at enzyme activity, in fact,

their enzyme is functional. The rate of pseudodeficiency varies throughout the world. It has high-frequency in East Asian populations, perhaps as many as 3.9%. Pseudodeficiency is associated with two specific mutations, however, and it can be affected by genotyping, so if you identified an individual through screening who had low enzyme activity levels, you would certainly want to genotype to ensure that it's not pseudodeficiency. And that of course assumes that there's not something else obvious that would point towards Pompe disease like cardiomyopathy.

The two other factors that you should be aware of is the issue with so-called "CRIM-positive," or "CRIM-negativity." So CRIM stands for "Cross-Reacting Immunologic Material." So, individuals who make some endogenous enzyme, even if it's not functional, are referred to as being "CRIM-positive." So they're making some enzyme, but it's just not working. If you are CRIM (+), then you're less likely to develop significant antibodies to enzyme replacement therapy. However, if you're CRIM (-) negative, you can develop high titers of antibodies that can potentially neutralize the enzyme-replacement therapy and eliminate its effectiveness as a treatment. There are different methods for detection of CRIM status, but the key thing here to recognize is that about 25% of the CRIM (+)

individuals can develop antibodies to enzyme replacement therapy as well, but it's usually not as significant as those who are CRIM (-), and we're going to return to the implications of CRIM status on enzyme replacement therapy a little bit later.

So the diagnosis is established based on low functional enzyme activity levels, by genotyping to rule out pseudodeficiency, to identify those who are carriers, to help predict infantile-onset versus late-onset disease, and also to predict CRIM status.

So, with that in the background, let's talk about the burden of disease in the United States. So overall incidence based on a number of different studies is expected to be about 1 in 28,000 newborns. About 28% of patients are expected to have infantile-onset Pompe disease, of which 85% would be classic Pompe disease, and about 15% then would be the non-classic infantile forms. And about 75% of cases of the classic infantile-onset Pompe disease are CRIM(+), so you know of course then that would mean about 25% of classic infantile patients are CRIM(-).

Similarly, in terms of late-onset disease, about 72% of cases are expected to be late-onset. And in the United States, pseudodeficiency is expected in less than 1% of births. However, there is likely to be some regional variation, and I

suspect that states (inaudible) regions doing newborn screening, that the epidemiology will be refined further.

So, this slide surmises the expected outcomes from clinically-detected case, so that is in the absence of screening. So, you can see here that the median age of onset for infantile-onset disease is about two months of age, with diagnosis coming from three months later. Without therapy, individuals can proceed rapidly to death, or requiring mechanical ventilation. Across all types of infantile-onset Pompe disease by 24 months of life, the survival and ventilator-free survival rate is quite low, you can see, it's about 5%. So clearly, a disease associated with significant burden.

Now, this summarizes what we know about late-onset Pompe disease, that symptom onset is typically around 28 years of life, with diagnosis about 10 years later, followed by death some 27 years after diagnosis. And you know, it's always hard when you talk about the expected outcomes of late-onset disease, because there's problems with ascertaining new cases. So it may be that there are individuals with late-onset disease who just aren't recognized, and therefore wouldn't appear in this data, as opposed to individuals with infantile-onset form, which are more likely to be impacted.

So, now let's talk about how effective therapy is. So, compared to historical controls, enzyme replacement therapy by a year, by 52 weeks, when given by six months of age reduces the risk of death by about 95%, and reduces the risk of death or invasive ventilation by 87%. It extends overall survival, so see overall survival at 36 months up to 72%, and overall ventilator-free survival at 36 months being 49%. And as we discussed before, the CRIM(-) status is associated with worse outcomes, and there's also lower survival if enzyme replacement therapy is begun after six months of age. However, going back to this CRIM(-) status issue, there are questions that have been developed for immunomodulation to reduce the development of antibodies.

So one of the complicated issues is around pre-symptomatic detection of late-onset Pompe disease. So, there are no trials of pre-symptomatic enzyme replacement therapy for individuals with late-onset disease. And so treatment decisions are based on a large variety of clinical findings, as well as laboratory findings that can be used as markers for muscle damage. There is always concern about early identification of late-onset cases, because of the potential risks of beginning therapy before it's needed. I will say, however, that there's evidence

from a randomized control trial of enzyme replacement therapy for symptomatic individuals, generally in their forties, that enzyme replacement therapy can improve respiratory status and motor function. So we know that individuals with late-onset disease can benefit; the issue is when to start it.

So, the effect of treatment begun after symptom development for those with late-onset disease might be limited. You can mention that if you begin enzyme-replacement therapy after individuals have already developed muscle damage, it's not going to as effective, because once you have muscle damage, that's there forever. The enzyme replacement therapy does not reverse muscle damage. So there's a biological argument to be made that treatment begun before symptom development might be better. And this is certainly borne out from biopsy slides, and those sorts of things. But again, we don't have direct evidence that that's the case, and you can imagine that this is going to be a difficult issue to resolve, because if you want to test the hypothesis about whether the pre-symptomatic therapy of individuals with late-onset disease modifies their disease course, it would be a prospective study that would take many, many years, and of course it's hard to develop a cohort that's big enough to test that directly, because you know, it is a rare disease.

So, in terms of take-home points about Pompe disease, remember that about one in 28,000 have Pompe disease. Most cases are late-onset. There's good evidence that early identification of infantile-onset Pompe disease compared to clinical detection improves outcomes. There's no direct evidence that pre-symptomatic treatment leads to better outcome; however, you can make that argument based on biologic plausibility. Most cases of infantile-onset Pompe disease are CRIM(+). CRIM(-) is associated with worse outcomes; however, immunomodulation can help.

So with that, that ends my presentation, and I would like to now pass the presentation on to Dr. Bodamer.

Dr. Olaf Bodamer:

Well thank you very much. I'm most grateful to the organizers for including me in today's webinar presentation.

I would like to start out by presenting one of the case reports, one of the cases I have seen back in Europe. This was an 18-year-old female who presented with a proximal progressive myopathy, was diagnosed at an outside hospital with Pompe disease. And I will just highlight some of the diagnostic

issues that arose from this case. The patient presented with elevation of creatine kinase (CK), moderate elevation. The muscle biopsy that was done by a neurologist showed vacuolar myopathy, and you see the different slides here on the top right-hand side, you see this vacuolar myopathy appearance. You see the positive PAS stain, and you also see a nice EM picture. Enzyme was then tested to confirm the late-onset Pompe disease in fibroblasts with some residual enzyme activity, and the diagnostic avenue was completed by molecular analysis of the Pompe disease gene.

Now there are several issues I want to highlight here. I mean, initially the clinical presentation was a moderate elevation of CK, should have already given kind of a suspected diagnosis of Pompe disease, so clearly the muscle biopsy was not indicated, what are you in this case, and confirmatory testing should have been done either on leukocytes, dried blood spots as I will show you in a moment, and obviously by molecular analysis.

So there are different diagnostic avenues we need to consider in the diagnostic stream for Pompe disease. Obviously, the initial suspicion should arise from the presence of clinical symptoms. We realize that there are general laboratory abnormalities that are present in patients with Pompe disease. There certainly, on

the muscle tissues, certain features, histological features, and histochemistry that can be utilized to come to a conclusive diagnosis, but certainly the gold standards are analysis of enzyme activity, of activity of the acid α-glucosidase, and molecular analysis of the respective gene.

So let me start out by talking a little bit more in detail about the histology and the histochemistry. Again, you see an example of a vacuolar myopathy in this panel here, with the Swiss cheese-appearance of the muscle. But you also see that different muscle sections are affected differently in this patient. So histologically, it gives you the appearance of a vacuolar myopathy. Vacuoles contain PAS(+), PASD(-), and contain acid phosphatase. The degree, however, of the pathologic change may actually vary, as demonstrated here, within the same muscle, from muscle field of almost from fiber to fiber. And it may certainly vary with different muscles, and with different clinical severity of the patient. We know that enzyme analysis in muscle tissues is feasible, but certainly not the preferred route for diagnosis, and I would actually argue that muscle biopsies in the primary diagnosis of Pompe disease is obsolete, as it may miss the diagnosis.

To highlight this fact, I will show you four different muscle biopsies that were recently published in a very nice overview on laboratory testing for Pompe disease. Panel A and Panel B show you muscle biopsies in late-onset patients with minimal myopathy with minimal clinical symptoms. Panel C shows you an example of a patient with a somewhat more severe clinical phenotype, whereas Panel D shows you a more severe clinical phenotype, and you see that there's marked difference between the different panels. Panel A and Panel B only show very mild myopathic changes with relative preservation of muscle tissues and structure, whereas Panel C gives you some indication of vacuolar myopathy as indicated by the arrows, and it's obviously more significant in Panel D.

So the take-home from here would be that muscle biopsy may play a role in the differential diagnosis of proximal muscular dystrophies, or proximal muscular myopathies, but if Pompe disease is suspected on clinical grounds, one should consider moving forward with enzyme analysis as the first-line test.

As I mentioned earlier, there are several general abnormalities. Certainly in the vast majority of patients, there's elevation of CK. It's a moderate elevation up to five times normal upper limit, so I rarely see patients with Pompe disease who exceed

1000U/L. I've seen end-stage late-onset Pompe disease patients with end-stage disease where there was very little muscle tissue left, and they have close to normal, or even normal CK levels.

There's also elevation of serum aldolase and transaminases. The ratio of AST and ALT typically is one; it's only moderately elevation of transaminases. But this may confuse, as sometimes, these elevations are seen initially without having a CK measured, and they may be consequently misinterpreted as liver disease, and we certainly have several referrals over the last couple of years where elevation of transaminase has led to a genetic referral, and the ultimate diagnosis was Pompe disease.

Slide 21:

This slide gives you an overview, again taken from the same publication, of different muscular dystrophies, myopathies that have a kind of a similar clinical appearance of proximal muscle involvement and proximal muscle weakness, and their respective, to expected CK levels. And you see kind of a fine line here that Pompe disease may have moderate elevations compared to some of the other more severe or significant elevations of CK.

The gold standard, as I mentioned, is analysis of α-glucosidase activity using two different laboratory techniques. One is the

fluorometric assay technique, that is a singleplex technique that is certainly useable for high throughput. It's ideal for dried blood spots and/or leukocytes, has been validated and is offered as a clear and tamper-proof test in several laboratories across the United States.

The second technology is tandem mass spectrometry. This is a technology that utilizes a Pompe disease-specific substrate, or enzyme-specific substrate in internal standard. The advantage of this technology is that it has multiplex capabilities. Again, it's suitable for high throughput analysis, and to my knowledge, only has been validated in CLIA and CAP for dried blood spots, but not yet for leukocytes. Somewhat of a disadvantage, depending on what protocol one uses, is the length of the assay time, which may take up to 36 hours due to the overnight incubation of the sample.

In any case, inhibition of isoenzyme maltase-glucomyalase by arcabose has to be incorporated into a protocol that utilizes a α-glucosidase activity irrespectively of the technique that one uses to avoid false negative results. There's nice correlation of enzyme activity in fibroblasts with phenotype, so late-onset patients typically retain some residual enzyme activity, compared to infantile-onset patients; however, this has not been

true for the most part when we look at α-glucosidase activities in dried blood spots and/or leukocytes. So the enzyme activity is not really a good predictor for phenotype.

Cross Reacting Immunological Material was also mentioned in the previous presentation, and highlighted there are the fact that CRIM(-)infants who lack the endogenous enzyme will mount an antibody response against the recombinant enzyme. So it may be worthwhile and may be important to determine the CRIM status, which can be done so in fibroblasts and/or peripheral lymphocytes using a Western blot technique, and I will show you an example on the next slide. The CRIM status, as mentioned earlier, may be however, predicted based on the genotype in the vast majority of CRIM(-) patients, and that percentage may exceed 95%.

To give you an example, based on the recent application, this is a picture of a Western blot where the B(+) and F(+) are CRIM(+) samples, either from blood or from fibroblasts, so you see four different controls at different concentrations, a serum protein standard ladder, and you see here on the right-hand column complete absence of those protein fractions in a sample of a patient who is CRIM(-). Again, this is based on fibroblasts.

The molecular analysis as the gold-standard, the gene spans about 28kb on chromosome 17, encompasses 20 exons. There have been more than 250 pathogenic mutations, and there's a nice resource is the Pompe Center in Rotterdam at the Erasmus University, where they have a very comprehensive database, listing most, if not all known pathogenic mutations in the GAA gene, in relation to the phenotype and about 75% allow us to predict the phenotype. There are some common pathogenic mutations in different ethnic populations, Caucasians, African-Americans, and the Chinese population, and the previous talk also highlighted the importance of pseudo-deficiency alleles that will lead to a significant reduction, apparent reduction of enzyme activity in the enzyme assays, but not lead to Pompe disease as a clinical manifestation of Pompe disease.

So a diagnostic algorithm to diagnosis patients with Pompe disease is obviously based on the clinical suspicion. Spectrum of disease, infantile- versus adult-onset disease. General laboratory abnormalities, general test such as a cardiac echo and x-rays, followed by enzyme testing, and now we would actually utilized dried blood spots and/or leukocytes for the most part. I need to emphasize, this needs to be the CLIA/CAP-approved laboratory, who has a significant sample load, so that

the requesting physician can be assured of the quality of the testing. Lower enzyme activity would be followed.

In our laboratory, we actually use the same dried blood spot to confirm Pompe disease by demonstrating two pathogenic mutations and obviously CRIM status is important in infantile-onset cases. However at that stage, we should not forget to evaluate organ manifestations and identify treatment goals as we will hear in the following talk.

So staging plays an important role as part of the diagnostic process. There are different blood tests, different investigations, an MRI angiogram in selected patients, quality of life, and so forth, and muscle function tests at baseline obviously are important if one considers enzyme replacement therapy.

So let me summarize. Diagnosis of Pompe disease has to be timely, to maximize the benefit of therapy. We know that there are general laboratory abnormalities that include a moderate elevation of CK and transaminases in the vast majority of patients. I would argue that muscle biopsy is obsolete for the diagnosis of Pompe disease, and the gold standard should be really analysis of the enzyme activity in either dried blood

spots or leukocytes, followed by molecular analysis of the gene, and obviously we have to appreciate pseudodeficiency alleles. And I would like, again, to highlight the fact that diagnostic testing should be done in an experienced laboratory with a sufficiently high sample load.

With that, I'd like to conclude, and I'd like to pass on the microphone to Neena Champaigne, who is the biochemical geneticist as Greenwood Genetics Center in South Carolina. Thank you.

Dr. Neena Champaigne:

Thank you for the opportunity for being able to present today on the topic of treatment, and we've already heard a little bit about treatment from the first two talks. And I'll be wrapping up today by spending a little bit of time delving into some of the current and future treatment options that are available for individuals with Pompe disease.

So, when we talk about treatment targets, it's helpful to look at the pathway, and look at where we may target treatment. So, as we've heard, Pompe is due to a deficiency of the enzyme α-glucosidase, and this deficiency results in a significant accumulation in several tissues, ultimately leading to

compromised cardiac, respiratory and muscle function. Up until 15 years ago, we had no specific treatments, and it was primarily focused on supportive care for the heart, the lungs, the muscle, and just overall quality-of-life. Looking at the pathway, one of the main targets and leading contenders that was immediately obvious was to replace the enzyme and exogenous lysosomal enzyme replacement is now, what we named ERT, or Enzyme Replacement Therapy. Along that lines, other targets to reduce the substrate of glycogen or chaperones to help the enzyme activity, and ultimately potentially coming up with a long-term restoration with gene therapy, which is something that has also been considered as an option.

So looking at the different articles that have been published over the years, today there have been over a thousand articles on treatment regarding strategies and outcomes for Pompe disease. The first article appearing just one year after the discovery of the enzyme deficiency of α-glucosidase in 1963. There were some initial studies early on that were unsuccessful, based on the concept or ERT, but in the 1990s, DNA technology allowed for large-scale production of recombinant human enzymes, and made it more of a reality, and a viable option for various lysosomal storage disorders, including Gaucher, Fabry, and Pompe disease. You can see here that there are upticks that coincide

with major time points in treatment options. And so in the early 2000s, late 1990s, there were the initial clinical trials that used this recombinant technology to start enzyme replacement therapy, and then with the FDA approval of enzyme replacement therapy in 2006, you see a significant amount of increase in publications.

Looking at the treatment strategies and how they're based specifically for enzyme replacement therapy, this figure illustrates the receptor-mediated uptake of lysosomes that provides this basis. So, in the upper-left hand corner, is a normal cell, or potentially a genetically-modified cell that depicts where the lysosomal enzymes are initially glycosylated in the endoplasmic reticulum, depicted by the green circle. They then are passed to the Golgi apparatus where they obtain a mannose-6-phosphate modification, indicated by the red circles, where they can then bind to the mannose-6-phosphate receptor, and be targeted to the lysosome. While this is the majority of the targeting, there is a small amount of enzymes that are secreted from the cell, and can also be bound by a mannose-6-phosphate receptor on an adjacent cell, also to be internalized from the cell surface, and subsequently targeted to the lysosome, and ERT takes advantage of this receptor-mediated uptake to target exogenous enzyme that is given intravenously to

make up the deficiency in these cells. And again, ultimately if we can genetically-modify with gene therapy, it would provide a more permanent target solution down the line, and we'll touch on that a little bit later.

So, looking at the initial clinical trials that were done that were the proof-of-concept for recombinant human GAA, two trials were published within a year of each other. The first one was based from a group in the Netherlands, published in 2000, and reported phase 2 trials that were conducted on four patients with infantile Pompe disease, and they used recombinant human α -- glucosidase from transgenic rabbit milk. These individuals were given weekly infusions over 36 weeks, and they were able to demonstrate that there was a significant improvement. Cardiac function improved dramatically. Motor function also improved, and all survived beyond a year, which previous to this had been unheard of, with most individuals dying before a year of age. They did note that individuals who were diagnosed below six months seemed to do better than older patients who were seven-to-eight months at the start of treatment. They also demonstrated that they were able to see some evidence of improvement by a normalization of α-glucosidase activity on muscle biopsy, as well as decreased glycogen material.

A similar study conducted at Duke, with three patients who were treated for one year with recombinant human GAA that was produced from Chinese hamster ovary cells were also reported a year later, and they had very similar outcomes, with improved cardiac function, but noted that there was more variable outcomes in terms of motor and respiratory function, but were able to demonstrate that overall survival beyond a year was obtainable for all individuals. They also noted a similar pattern on muscle biopsy with improved α-glucosidase activity and some variable glycogen material.

Through these studies, they learned that this treatment was generally well-tolerated, but there were some individuals who had minimal allergic reactions that they could address with antihistamines and slower infusion times, and it appeared that individuals with more advanced disease, and more extensive muscle disease, as we've reviewed in the previous two talks, that was beyond repair, seemed to be more resistant to treatment, as well as antibodies that were generated in two patients, seemed to demonstrate decline in their outcomes.

So, over the past 10 years, these multiple clinical trials have demonstrated the improved survival rate, the improved ventilation-free survival rate, improvement in cardiac and motor

function, as well as demonstrating that the age of onset, stage of disease, and CRIM status are all important aspects of treatment response.

We've heard about CRIM status for the early-onset infantile form, and this impacts approximately 20% of infantile-onset form, where they have negative status due to no endogenous GAA enzyme produced. This then results in these high-sustained antibody titers that essentially neutralizes and impairs the effectiveness of ERT. These individuals typically demonstrate approximately four-to-eight weeks of improvement, and then have a decline with reduced survival, decreased cardiac response, and also requiring ventilator usage after a period of time.

In order to address this specific group, which encompasses both the negative status, as well as 25% who do have a positive status, other strategies for treatment have been developed, referred to as "immune toleration induction." And so this approach is used to prevent or eliminate immune response that occurs to the recombinant human GAA. A number of strategies have been looked at, and use a combination of these various medications, including rituximab, intravenous immunoglobulin, and methotrexate. The strategy involves suppressing both B and T cells, and then exogenously replacing some of the immune

capacity to get them through the high-titer period. There's also been some suggestion that gene therapy may be another option for some individuals, in which it allows for some immune toleration if introduced early on. So other strategies may be helpful in dealing with this CRIM status in immunologic response.

So we've reviewed that early diagnosis is important in terms of impacting the course of treatment, and a group in Taiwan has the ability to start newborn screening, and reported their early findings from newborn screens in October 2005 through December 2007. With the 200,000 newborns that were screened, they identified six cases of infantile-onset Pompe disease, that they were able to quickly diagnose and treat, all by one-and-a-half to three-and-a-half months of age. After being on treatment for 14-32 months, they were able to determine that these individuals had normal cardiac size, respiratory status, and motor development, again highlighting the importance of early detection and treatment.

We have reviewed, late-onset Pompe disease is slightly different. There are not as many studies, and there are not any prospective studies, and the initial strategies did note that while there were improvements, most of it was mostly a gain of

stabilization and minimal improvements in both respiratory and muscle function, that overall, general improvement in quality of life. And, as Dr. Bodamer mentioned, a major part of the response rate depends on the stage of the disease, so once the muscle disease is advanced, then ERT becomes less efficacious.

One other factor that was identified is that the targeting for the mannose-6-phosphate, there are fewer within the skeletal muscle, and so this may also impact the targeting of enzyme for this particular subset of individuals with Pompe disease.

A consensus group met to review the data on late-onset Pompe disease, and they were able to make some recommendations regarding when the treatment should be initiated, particularly since more individuals are anticipated to be diagnosed either through newborn screening, or siblings that are diagnosed. And, their recommendation was that testing should be performed, and treatment should be initiated once decreased pulmonary function, or decreased muscle weakness has been determined. After they were starting on treatment, this efficacy should then be reassessed after a year to determine if there were improvements, either in slowing, reversing, stabilizing, or preventing various symptoms that can ensue from the late-onset Pompe disease.

This concept was further expanded on the study of newborn screen in Taiwan. So over a four year period, they were able to screen 344,056 individuals. Thirteen cases met the criteria for late-onset Pompe disease, meaning that they had no cardiomyopathy. Four of these cases were noted to have some muscle involvement with low muscle tone, developmental delays, as well as some biochemical evidence with elevated creatine kinase, and they were ultimately started on ERT with improvements in their clinical symptoms. They have nine other untreated cases that they continue to monitor every three-to-six months to determine whether or not they need enzyme replacement therapy, and they will continue to monitor them.

Looking at enzyme replacement therapy, there are two forms that are currently FDA-approved and were reviewed in the first talk. It's important to note that one is for infantile Pompe, and the other is for individuals greater than eight. This enzyme replacement is every two weeks, and the dose is quite high compared to other enzyme replacement therapies, which are closer to 1mg per kg per dose. The treatment can be very costly, and the prices here are listed, so an individual, an infant or a young child, the annual cost for the medication alone is $50-400,000. The cost for an older individual $300-600,000 per

year, and again does not cover the cost of administration or any other complications associated with enzyme replacement.

As we have reviewed, ERT does have several consideration and limitations, and it's certainly not curative. Some of the issues that we've talked about are infusion-related reactions, antibody formation, the difficulty of accessing and delivering the enzyme replacement therapy to the muscle tissues. In addition, there are increasing reports that there is an emerging neurological phenotype that has been noted, and this is also thought to be due to the difficulty of targeting neuronal cells, and the increased amount of glycogen that is accumulating within the central and peripheral nervous system, and ultimately the burdens of lifelong treatment, and the cost that ensues from this lifelong treatment.

So, in order to address this and try to improve on currently available treatment, there are certain strategies that are being employed. The first is to look at second-generation ERT, and there are two companies that are looking at this option. BioMarin currently is in phase 1 and 2 of clinical trials with an altered GAA that uses an alternative lysosomal targeting system with Insulin-like Growth Factor 2 (IGF-2), and the hope is that it can target to the muscle tissue in a more efficient

way. Similarly, Neo-GAA is using a synthetic bis-mannose-6-phosphate that's linked to GAA, and it is also modified to increase or enhance the receptor uptake. And this is an early clinical trial.

Another method for addressing the deficiencies or the efficiency of the enzyme replacement therapy is the use of chaperones. Chaperones are pharmacological agents that stabilize and rescue a misfolded or unstable protein. One such agent, the N-butyldeoxynojirimycin, or NB-DNJ, has been studied in fibroblasts, and it did demonstrate improved GAA transport to ER and lysosomes, as well as increased GAA activity. And there is one current phase 2 clinical trial with one such medication, duvoglustat hydrochloride, and this is being administered one hour prior to ERT to see if this will improve the efficacy and delivery of enzyme replacement therapy.

One other strategy that has been evaluated for a number of years is gene therapy. So progress continues to be made in this area, and several proof-of-concept trials have been performed in mouse models. The leading virus that has the lowest immunogenicity and improved targeting is adeno-associated virus. Trials have been done in Pompe knockout mice, and it has been determined that targeting to the liver appears to allow for more efficient

production, and more systemic effects, but similar issues with high antibodies were encountered in this target group as well. One other group has looked at addressing some of the peripheral nerve issues related to functioning of the diaphragm, and injection into the diaphragm seemed to restore some of the phrenic nerve activity, improve ventilatory function. Along that line, human trials that are in phase 1 and 2 are currently in progress by a group at the University of Florida, looking at another potential.

Lastly, prior to ERT, individuals did demonstrate that there were some strategies for substrate reduction and improved muscle function using nutrition and exercise. This could be another adjunct to ERT therapy, particularly for the late-onset group. A low-carbohydrate diet and a high-protein diet can minimize some of the glycogen accumulation, and also increase the increased overall muscle protein synthesis. And the aerobic exercise is used to increase the number of type I fibers, which use more of a fatty acid oxidation pathway for their energy production.

Lastly, as we have alluded to, this is a multi-system disease that requires supportive treatments from a variety of multi-disciplinary groups in order to address some of the physical and

speech issues that continue to ensue. They require frequent cardiac, neurologic and respiratory monitoring, as well as attention to nutrition and diet.

And lastly, ERT has been extremely helpful, and has resulted in dramatic improvements, but I think that we can all agree that a lot of critical work remains to be done. There are special patient groups that have been formed both in the United States and internationally that are available to address the ongoing needs that are necessary for this patient population. And in addition, the Pompe Registry which was established in 2004 is an important tool that clinicians and families can use to gather, track, and monitor data, to not only assess the natural history, but also look at the outcomes for ongoing and emerging treatments in this patient population.

With that, I'd like to wrap up and go ahead and pass over to, I think Patty for questions and answers. Thank you.

Patricia Hunt:

Thank you very much to all of the speakers for the wonderful presentations. Operator, can we open the question and answer section now?

OPERATOR:

Yes, ma'am. If you would like to ask a question, please press "*1". Remember to unmute your phone, and record your name clearly when prompted. One moment for the first question.

Patricia Hunt:

And while we are waiting, I believe there is one question that has been asked by the audience, and I'm going to read it briefly. Has Secretary Sebelius added Pompe to the Recommended Uniform Screening Panel, and is there a reason why she did not? And I believe, I can go ahead and answer that question, and please correct me, participants, if I misrepresent. But I believe that the secretary has forwarded the recommendation to another internal committee for their review and approval. So she has not approved the recommendation yet, but I think soon will. Do any of the panel members have additional information they would want to add to this response?

Dr. Alex Kemper:

Yeah, this is Alex, just to say that that's correct.

OPERATOR:

And again, this is the operator. If you have a question, please press "*1" on your touchtone phone.

Patricia Hunt:

And while we're waiting, another question is, for the speakers, do you expect access to simple multiplex enzyme activity assay will change the algorithm for diagnosis? I'll repeat the question; do you expect access to simple multiplex enzyme activity assay will change the algorithm for diagnosis? (pause)

I don't know if any of the panel members have a response to the question.

Dr. Alex Kemper:

I guess -- this is Alex -- I'm not entirely sure if I understand the question, but I think that whatever the screening process is, the current state of the science wouldn't change the diagnostic algorithm that would occur after a positive screen.

Patricia Hunt:

OK, thank you very much. Another question is what source is used for the incidence of the one to 28,000? I think in one of the --

Dr. Alex Kemper:

Yeah, that was me again. There are a variety of different sources that ultimately give rise to that, that include the anonymous dried blood spot study that was done by Dr. Ron Scott. It does not incorporate all the numbers that have come out of the real-life screening experience in the state of Missouri, and that's because at the time that we developed our report, those numbers were still preliminary. And, I don't know if anyone else wants to comment about that.

Dr. Neena Champaigne:

I would just agree, and it just depends on the population, and I think that's a moving target as we embark on newborn screening, in terms of determining the incidence.

Dr. Alex Kemper:

That's a really good point, that whenever you start doing population level screening, you find that the incidence is different than what you expect, because there are cases that otherwise might not come to light. And, inevitably, the spectrum of disease also broadens.

Patricia Hunt:

Great points. Operator, do we have any calls?

OPERATOR:

No, ma'am, we do not have any calls in the queue at the moment.

Patricia Hunt:

And an additional question is what percentage of CB and IO, and what percentage of pediatric, I guess, and infantile forms, and I guess what percentages, and how easy it is to diagnose between the different categories? I think that's late-onset and infantile-onset, and how difficult is it to diagnose the difference.

Dr. Alex Kemper:

This is Alex; again, I'm going to defer to the other speakers who have more practical hands-on experience with that process, you know. But the evidence strongly says that with genotyping, you can fairly well predict whether or not an infant is going to have infantile version or the late-onset version. And remember too that the most severely affected newborns with Pompe disease may also have other findings, such as cardiomyopathy.

Patricia Hunt:

And next question I think is going to be pretty much the same response. The question is, if newborn screening identifies a

positive Pompe result, can a confirmatory test distinguish between the infant-onset and late-onset form?

Dr. Alex Kemper:

Yeah, so again, it really hinges on genotyping. And I don't know if Dr. Bodamer probably can comment on that further. Olaf, if you're on the line, your phone is on mute.

Patricia Hunt:

He is not on the line, he had to leave.

Dr. Alex Kemper:

OK, all right, but then he can't comment on it. But I think, you know, the crux in it is genotyping.

Patricia Hunt:

Operator do we have any questions?

OPERATOR:

No, ma'am, we have no questions in the queue. If you'd like to ask a question, "*1" please.

Patricia Hunt:

I think we'll wait a few more minutes to see if we have any other questions submitted, but in the meantime, I'd like to thank all of the speakers for the wonderful presentations, and thank the audience members for participating today. It's been a very good session. Should we just wait a few more minutes to see if we have any more questions, or --

OPERATOR:

I think second at this point, and reminding folks about the second part of webinar, which you're going to be moderating as well, will be important.

Patricia Hunt:

Once again, thank you for participating, and next week, next Wednesday, February 26th, same time, to 12:30 PM Eastern Time, we will have the second part of this webinar series that will review the states' experience in implementing Pompe disease screening and methods being utilized in their laboratories. And so that should be an exciting session as well with some great speakers lined up, so please join us next Wednesday, and I believe the remainder will be sent out with a link for that web conference part two.

I am not seeing any additional questions out, so.

Dr. Alex Kemper:

So get out.

Patricia Hunt:

Thank you very much everyone for participating, and thank you for joining us today, and we look forward to next week. Thank you very much.

OPERATOR:

This is the operator. This conference has concluded. Feel free to disconnect at this time.

END OF AUDIO FILE