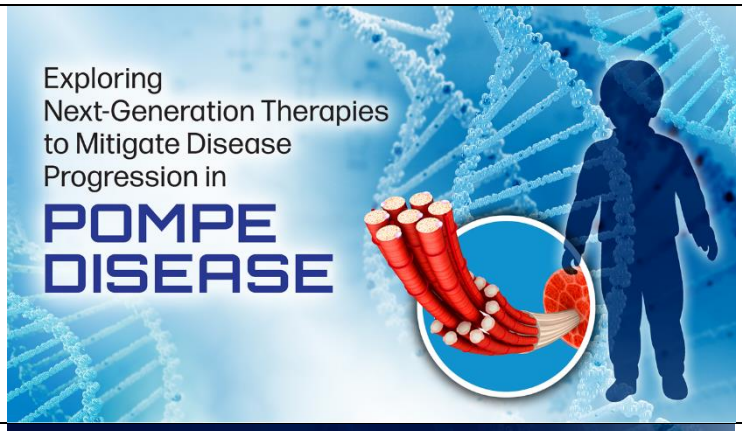
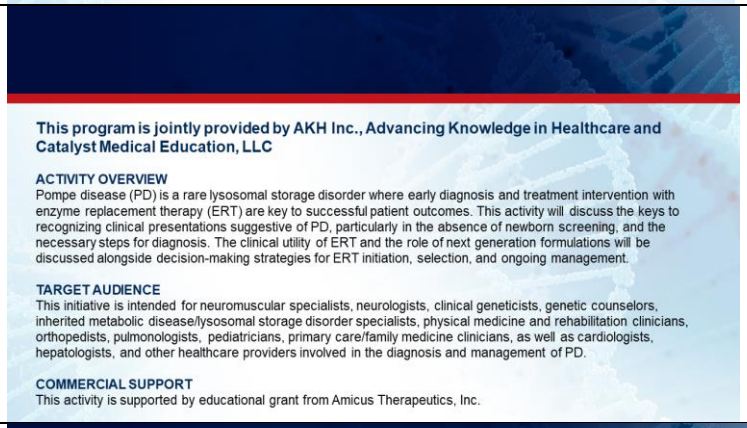
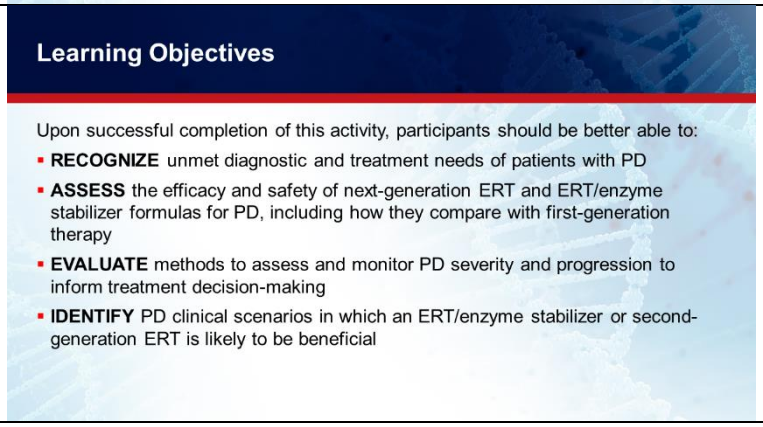
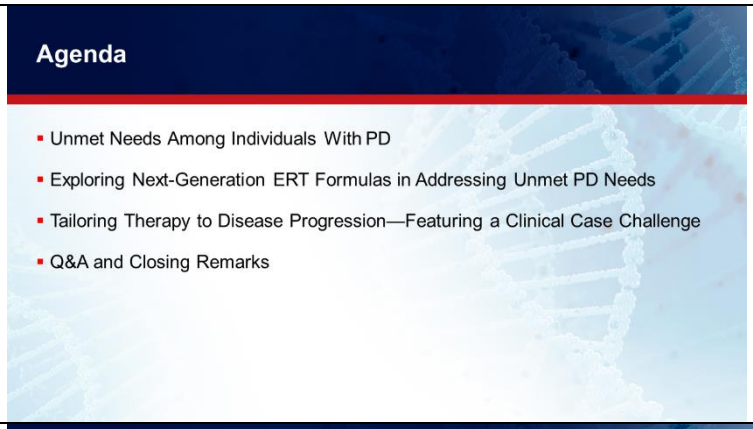
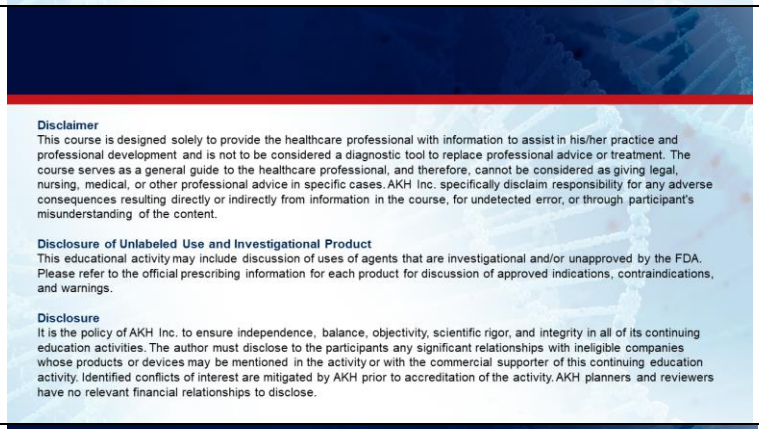
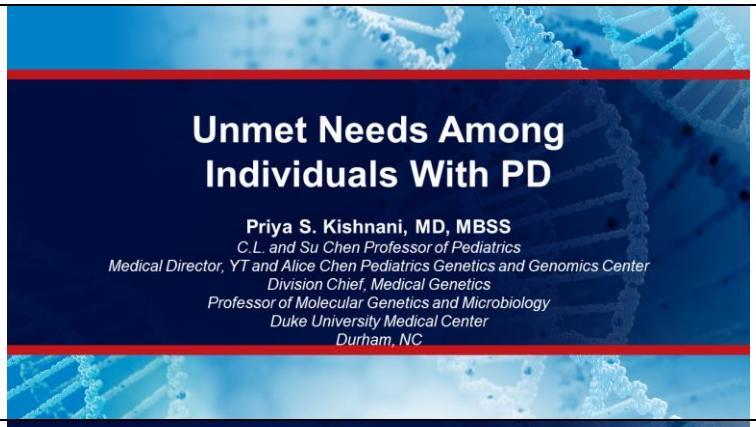
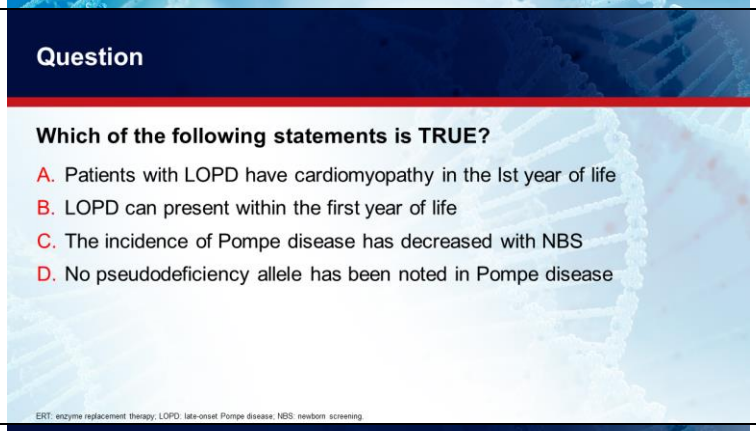
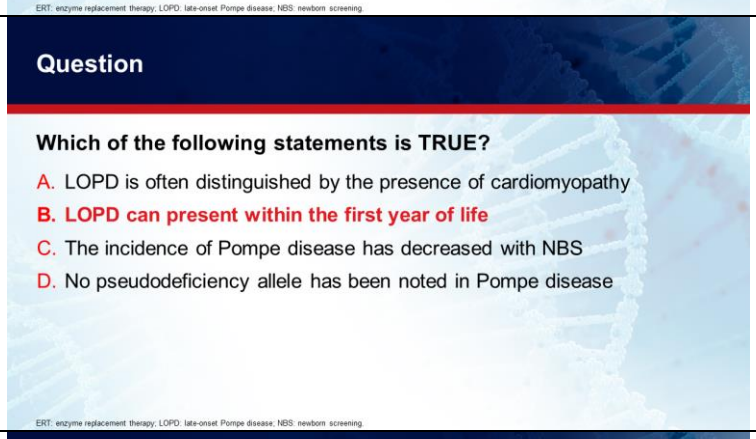
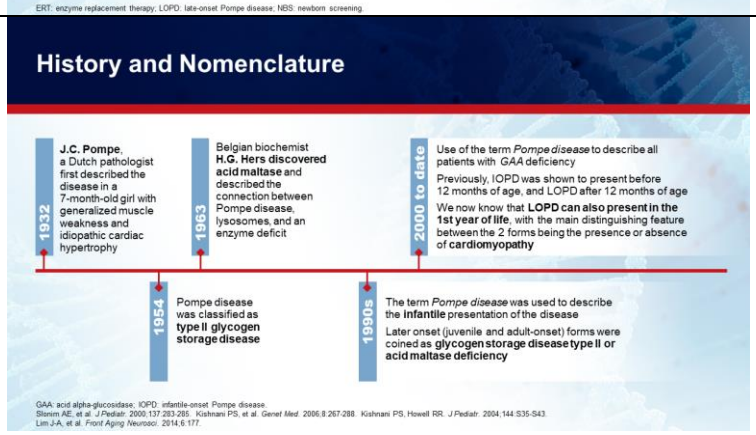
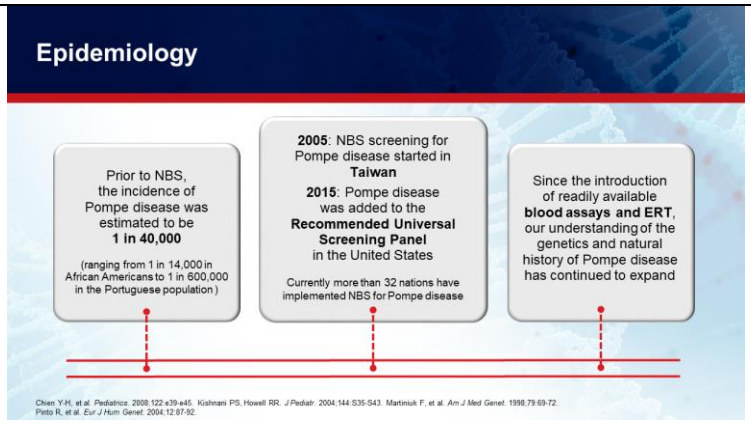
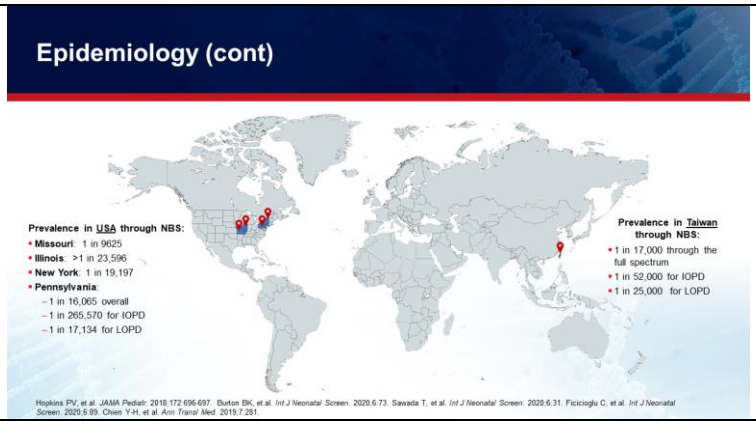
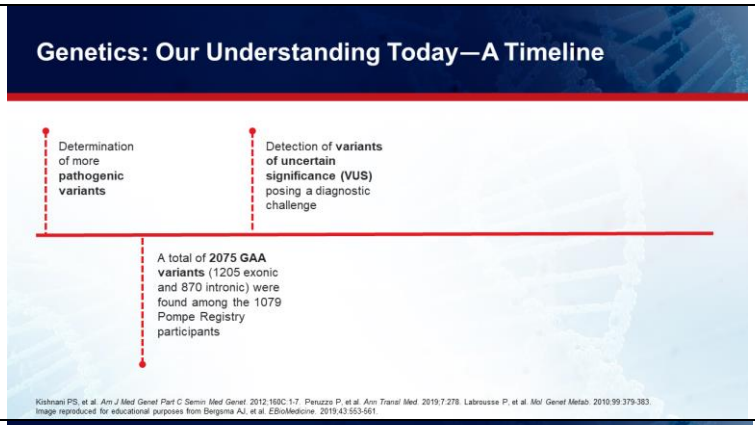
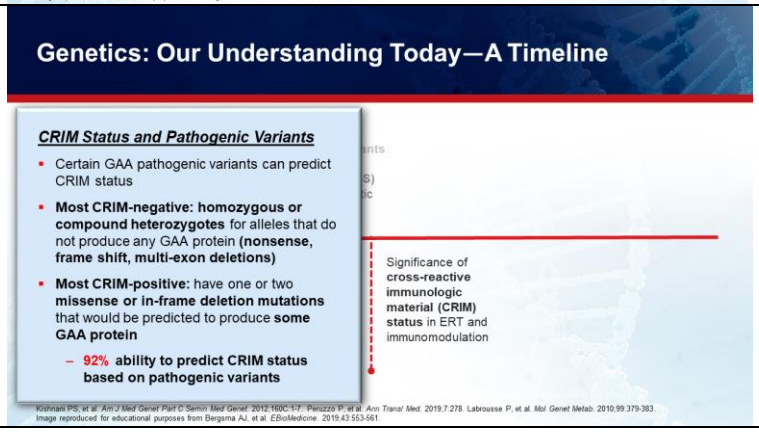
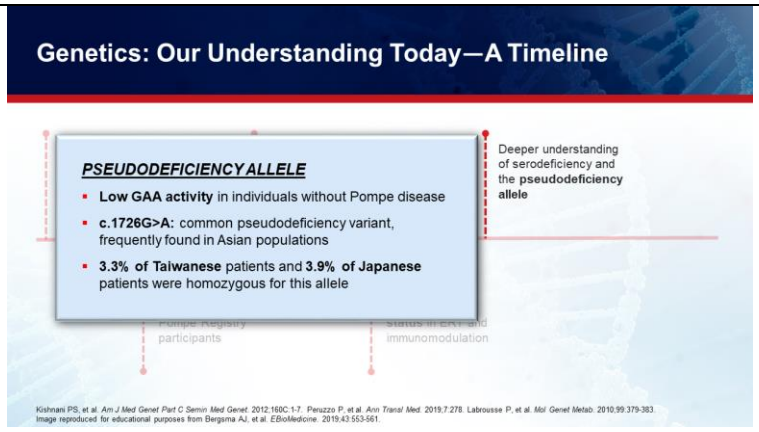
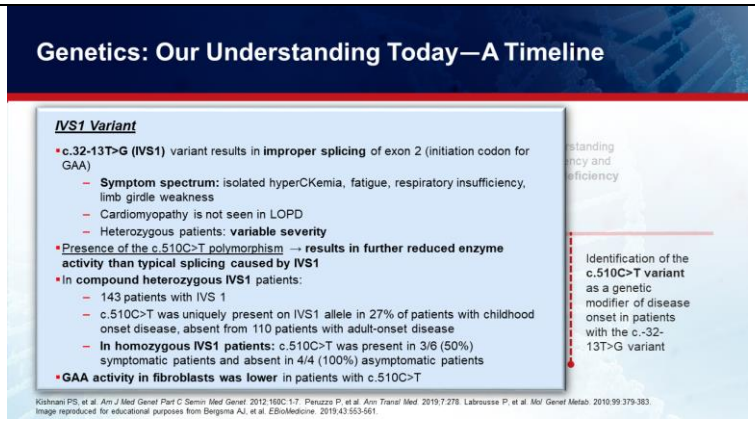
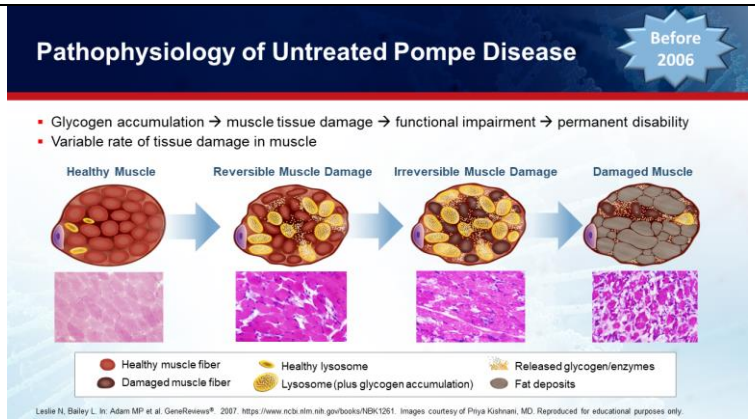
1		**Priya Kishnani:** So, I think what we're going to be talking about today is next-generation therapies to mitigate the unmet needs in Pompe disease.
2		And I am really honored to have alongside with myself, my name is Priya Kishnani and I'm at Duke University, to have Professor Benedikt Schoser from Munich, Germany, he's a professor of neurology, and Professor Antonio Toscano from Messina, Italy, also a professor of neurology.
3		And with that, I'm going to get started to state that this has been provided by an educational grant from Amicus and is being done by Catalyst Education.
4		And these are the learning objectives.

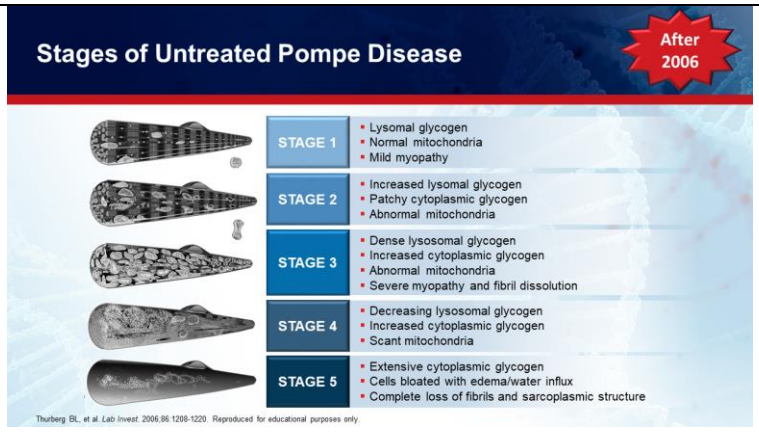
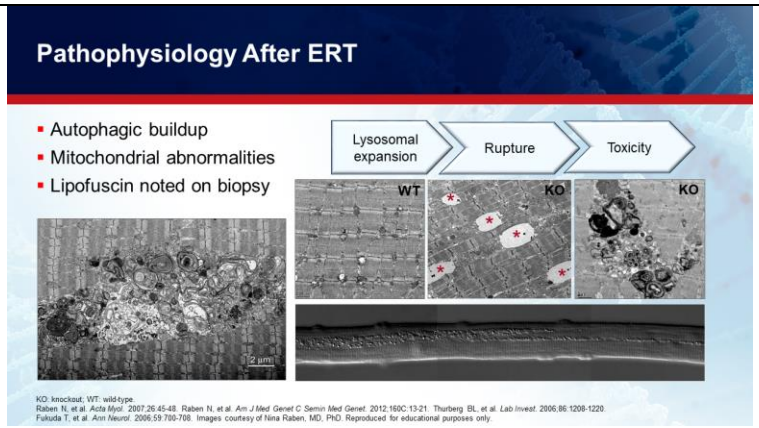
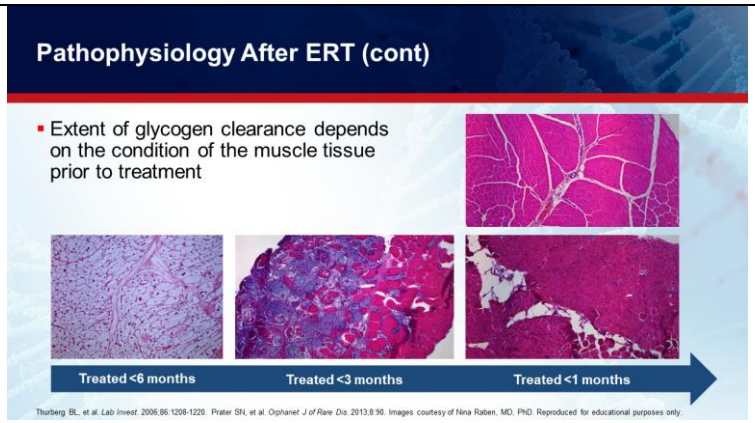
5		This is our agenda.
6		
7		
8		Toscano disclosure – **Prof. Antonio Toscano,** has received honoraria for advisory boards from Amicus, Bayer, Sanofi Genzyme, and Spark Therapeutics; honoraria for teaching activities from Amicus, Sanofi Genzyme, and Spark Therapeutics

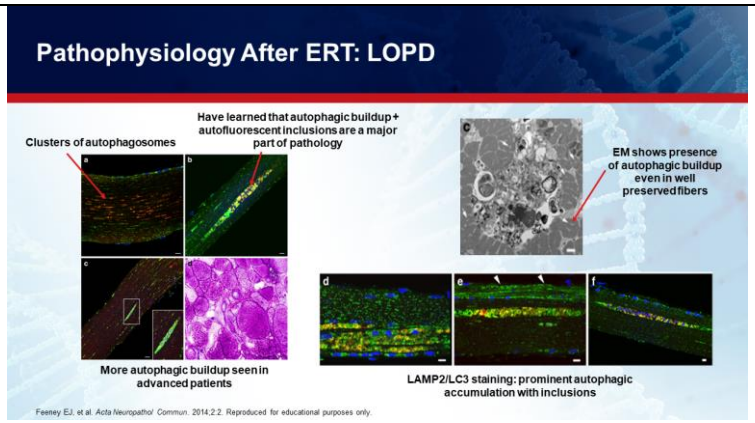
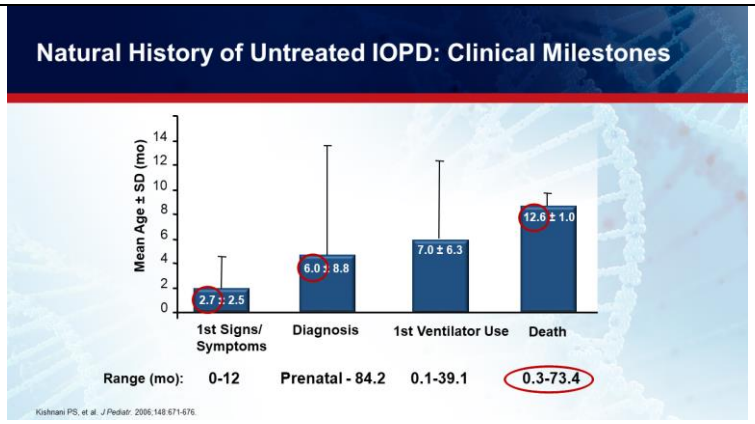
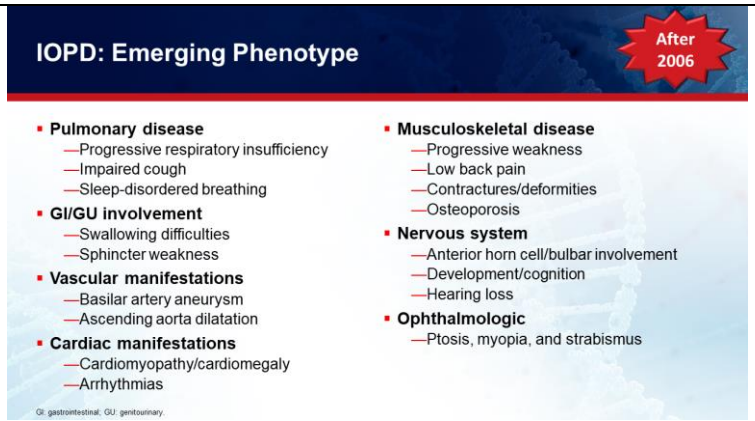
9		
10		
11		
12		And I will get started. So, I really thought I wanted to put in perspective today, from then to now, right, starting with the nomenclature. And just to start with saying that in 1932, when J.C. Pompe, who was a Dutch pathologist, described Pompe, it was for infantile Pompe disease. And then through the years, there were different nomenclatures that were given, like acid maltase deficiency, glycogen storage disease type 2. However, from the year 2000 to now, the use of the term *Pompe* is to describe patients across the disease continuum. And previously what we knew, and we still know that today, that infantile Pompe is those who present

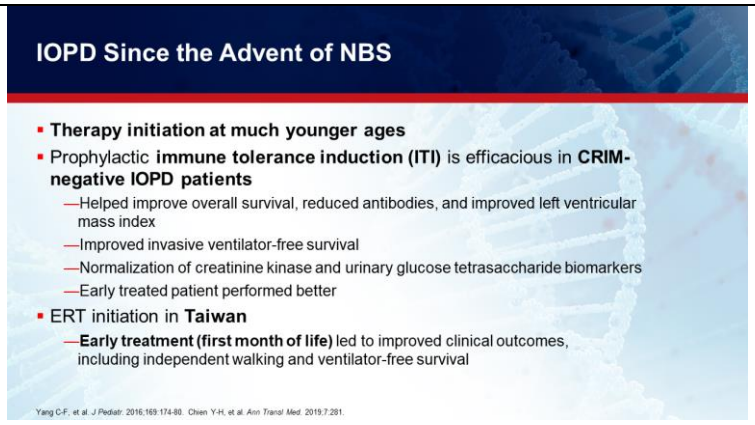
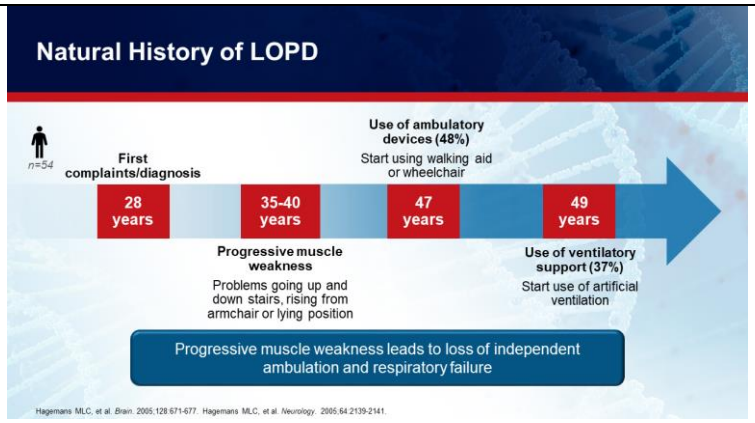
		prior to 12 months of age with cardiomyopathy. What we thought was late-onset Pompe disease spresented after 12 months of age. I think today we know, with the knowledge from newborn screening and through clinical cases, that patients with LOPD can actually present also within the first year of life. The main distinguishing feature is the absence of cardiomyopathy in LOPD in the first year of life as compared
13		to infantile Pompe disease. Epidemiology from then to now: Prior to newborn screening, the estimated incidence was about one in 40,000 and then with newborn screening, which started first in Taiwan in 2005. And then, in 2015, Pompe was added to the Recommended Uniform Screening Panel. There are about 38 states now screening for Pompe in the US. And what we are learning since that addition of newborn screening, but also because of the availability of blood spot assays and the availability of enzyme therapy, I think the epidemiology as well as our understanding of genetics has changed.
14		So, let's look at what we learned in Taiwan. The prevalence is about 1 in 17,000 across the disease continuum and in the US based on different state data, it can be anywhere from 1 in 9000 to about 1 in 25,000. So once again, a changing landscape in terms of epidemiology.

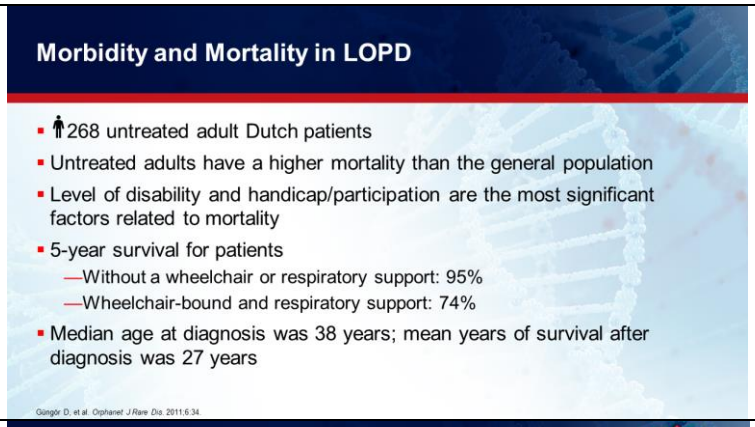
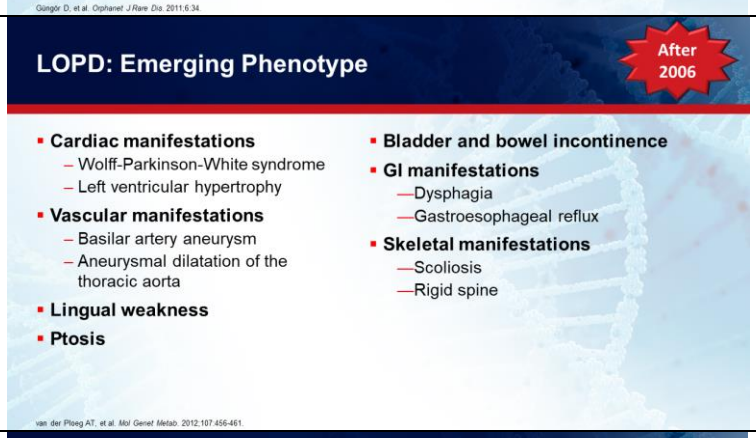
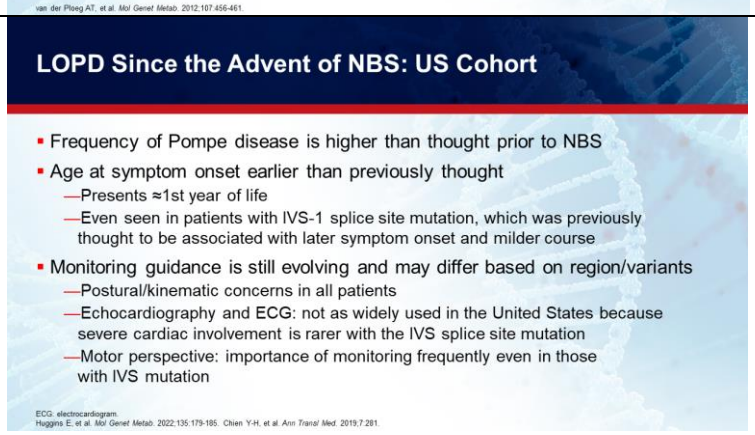
15		Let's talk now about our genetics understanding. Before 2006, we knew it was due to two pathogenic variants in the *GAA* gene. At that time, what we knew was 120 pathogenic variants. Then we knew mutations by certain population types. The *R854X* very common amongst the African American. Certain pathogenic variants amongst the Chinese. Then we also knew that infantile was more common amongst individuals of Chinese descent or those from Taiwan, also those of African American descent. And that late-onset Pompe disease had a higher incidence in the Netherlands of about a frequency of 1 in 57,000, and that the common leaky IVS splice site mutation was present in approximately 50% to 60% of cases with LOPD.
16		What do we know today? Definitely that there are many, many more pathogenic variants. And in a paper from the registry, what we were able to identify was a total of over 2000 *GAA* variants and this was recently published. The next what we learned is a new challenge, which is the variants of uncertain significance, which really becomes a big issue in the setting of newborn screening.
17		We also learned about CRIM status and that you can now do CRIM status evaluation beyond a Western blot and that today there is an ability to identify a patient, whether he or she is CRIM-positive or -negative in about 92% of cases in the setting of known pathogenic variants.

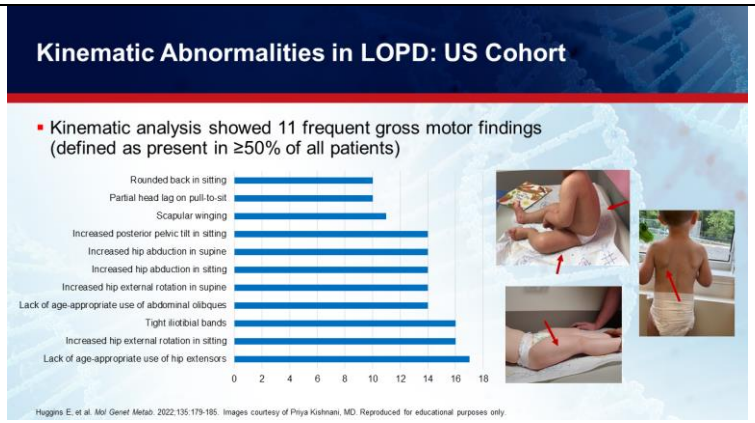
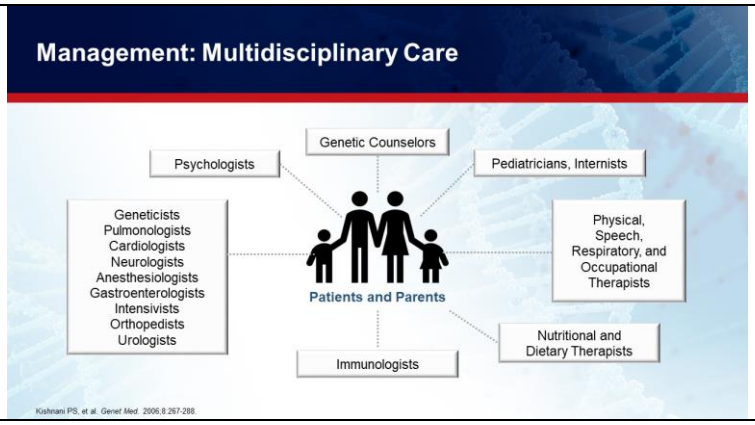
18		We also learned about the pseudodeficiency allele. Again, it becomes very important in the newborn screening setting, but also in the clinical setting. We all know this is low enzyme activity in individuals without Pompe disease. And we know that there is this common pseudodeficiency variant, the c.1726G>A, which is very common in the Asian population. 3.3% of Taiwanese patients and about 4% of Japanese patients are homozygous for this allele.
19		And then we've also learned more about this IVS variant. In the past, we said it was about 50% to 60% of patients of Caucasian descent have this variant. Today, we know it's upwards of 60% to 90%, and I think we are also recognizing that there could be certain polymorphisms which could modify splicing even further. And so today we know that there is the c.510C>T, which, if present, *in cis* with the IVS variant, alters splicing even further, reduces the enzyme activity even further, and if present, then the likelihood of the patient presenting earlier is more. However, the absence of the 510C>T does not mean a patient cannot present earlier, again telling us that there could be other variants.
20		All right, let's talk about the pathophysiology of untreated Pompe disease. Before 2006, this was our understanding. It's a lysosomal disease. There's buildup of glycogen over a period of time. There's seepage outside the lysosome, and there you go from a point of reversible muscle damage to completely damaged muscle. And if you look at the histology, below, it goes from what could appear like normal muscle to then completely damaged muscle.

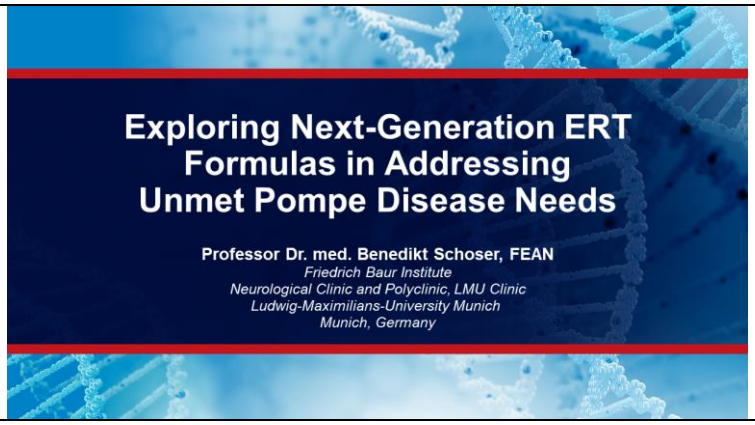
21		What have we learnt since then? After 2006, we started staging the muscle biopsy evaluation. We knew that once the glycogen was intralysosomal and there was just some seepage out, we called it stage 1 and stage 2. So, the earlier stages of the disease. Then we realized that as there's more extravasation of this glycogen, there's more advanced disease, and there could be some involvement of the mitochondria and then you go to end-stage disease.
22		What have we uncovered since that time? Since the ability of evaluating and the value of the muscle biopsy, which I'm going to try and reiterate again today, is, we realize that this is also a defect of autophagy and there can be autophagic buildup, which results not only in perpetuating the pathophysiology, but also in actually trapping the enzyme and its inability to reach the lysosome. We definitely know that there can be mitochondrial involvement and we also know that you can have lipofuscin, which is end-stage damage on muscle biopsy.
23		We also learned that, depending on the stage of the muscle and the muscle fiber, the amount of glycogen that is cleared is very much dependent on that, and the hope is that we go towards the less than age 1 month for infantile Pompe, and for a later onset to start at an earlier stage because your response to therapy is so variable and dependent on the stage of the muscle at baseline.

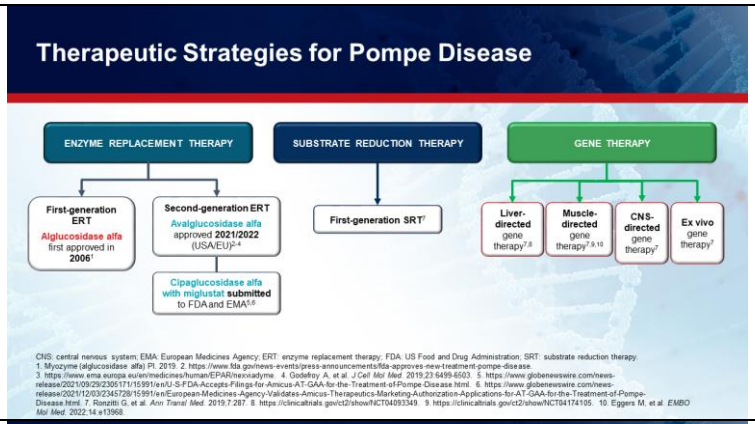
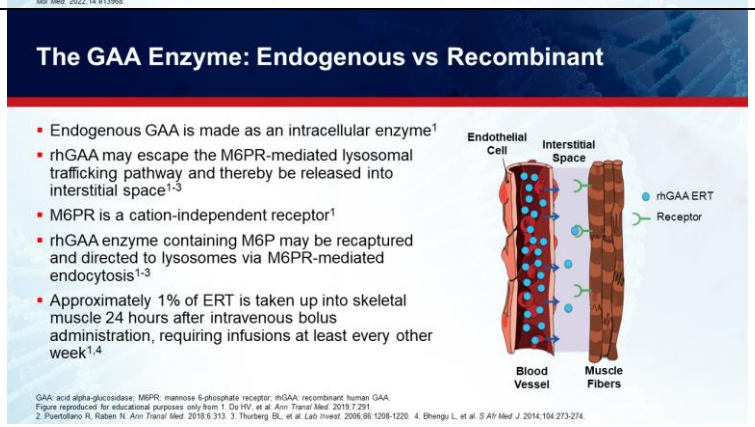
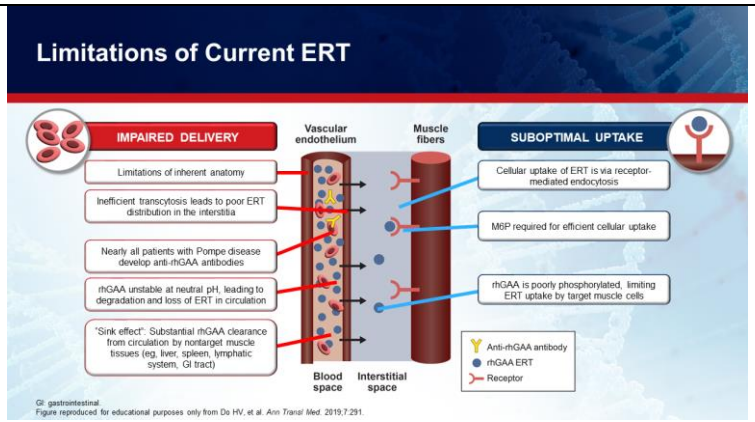
24		Again, this is beautiful work from Nina Raben, showing that even in the early stages of late-onset Pompe disease, there is a lot of autophagic buildup and that EM shows the presence of this buildup even in well-preserved fibers. So, I think the key to understanding is that the disease progression is occurring at a cellular level before there could be onset of overt clinical symptoms that we are currently evaluating.
25		Moving gears. What is the natural history of untreated infantile Pompe disease? We've seen this. This was a study that was done as a parallel, natural history study with the first clinical trial for ERT. At that time, we said first symptoms and signs was 2.7 months, diagnosis was at the mean age of 6 months. But look further to the right. What you see is that there are babies who survive past the first year of life, which we know today is nonclassic infantile Pompe disease. Again, very important in the counselling perspective, not every baby has classic infantile. It is a disease continuum, even amongst babies with infantile Pompe disease.
26		With therapy, what we've seen is that there's an emerging phenotype. Beyond the pulmonary and beyond the skeletal muscle, we know that there can be GI involvement, there could be sphincter incontinence, there could be vascular manifestations, there could be a persistence of cardiac arrhythmias because of glycogen accumulation. And now we know that there could be nervous system involvement because of glycogen in the anterior horn cells in the cerebrum, and also we've started seeing features of it in terms of hearing loss and foot slapping gait.

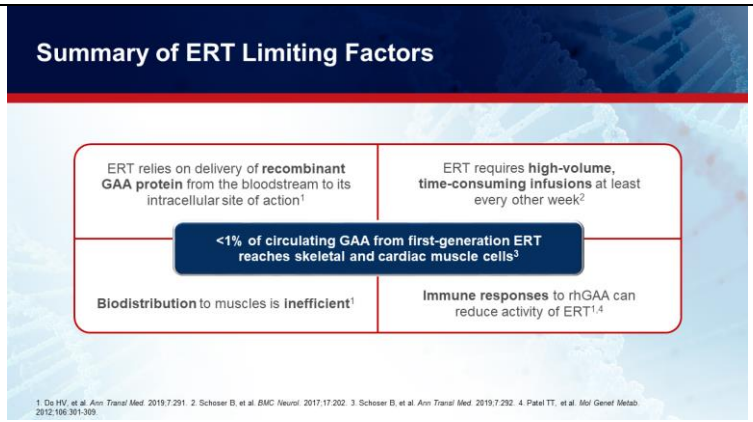
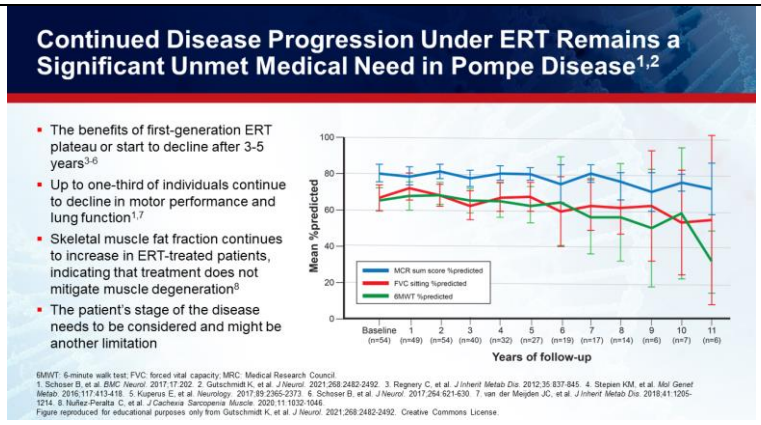
27		Now what happened? Since the advent of newborn screening, I think we've definitely recognized that presentation can be right at birth; in fact, it can be in utero, and that therapy initiation at a younger age is much needed. Even in the setting of CRIM-negative infantile Pompe disease, you can completely impact and change the outcome if you started early, if you did immune modulation and what you can see is that there is improvement in terms of babies able to walk, a normalization of the CK, the urine Hex4, and these patients are doing much better, much better. But the jury still is not determined, we've got to follow these babies long term. Also, data from Taiwan, the same message of course, came in much earlier, but this was in CRIM-positive babies. And in that data from Taiwan, the message really is that days matter in terms of when you initiate therapy.
28		Let's now switch gears to late-onset Pompe disease. This is the classic information that we have, first complaints and diagnosis at 28 years of age. A decade later, this progressive weakness. A further decade later, now there's much more use of walking devices and use of a wheelchair is possible, and also the use of ventilatory support. We know that there's progressive weakness, which leads to a loss of independent ambulation and respiratory failure, and also that there's earlier mortality in patients who are untreated with late-onset Pompe disease.

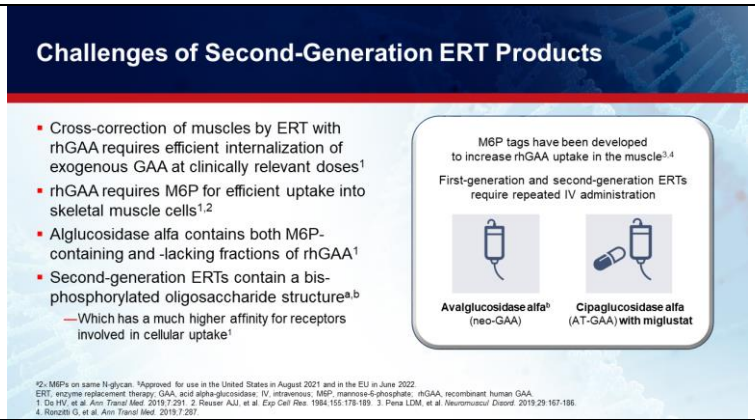
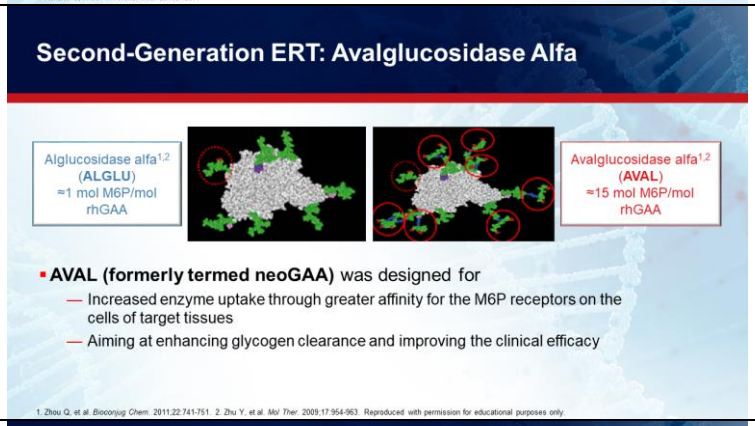
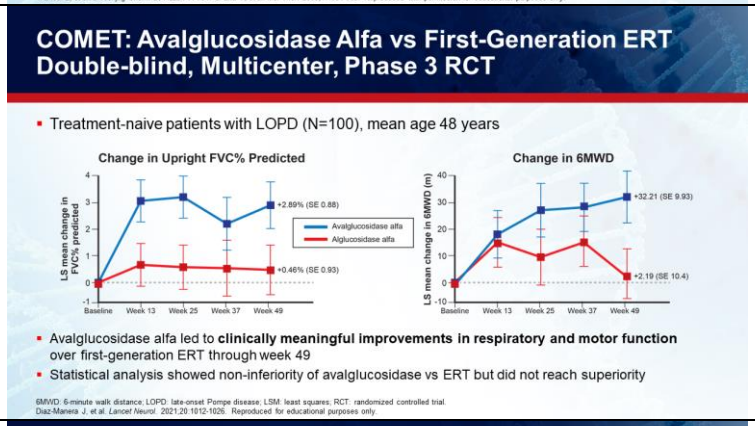
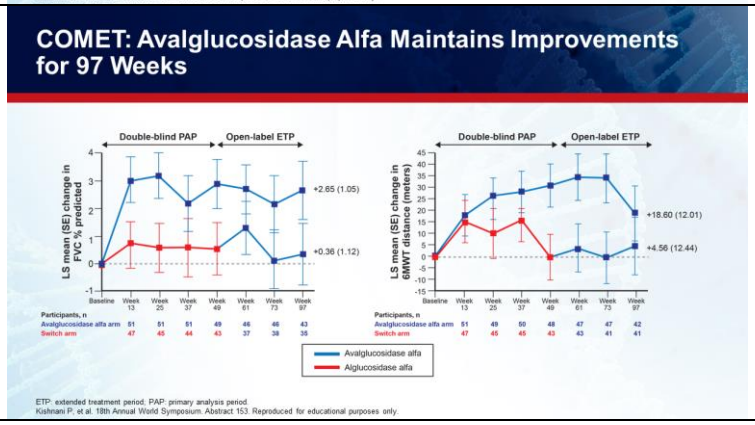
29		
30		This is the emerging phenotype, once again, beyond the limb–girdle muscle disease, beyond respiratory insufficiency, there can be cardiac manifestations, bladder involvement, skeletal manifestations, tongue involvement, and also nervous system involvement is now being recognized for late-onset Pompe disease.
31		What are we learning now? Since the advent of newborn screening for late-onset Pompe disease, and I think this has been a real eye opener, I've already talked about that the frequency of Pompe disease is higher than what was thought before newborn screening. We know that the age of symptom onset is earlier than which was previously thought, but how early is early? We definitely know that many babies are presenting in the first year of life, and even those babies with the IVS splice site mutation, which we know is the vast majority of patients we see in the clinic post newborn screening in the US, is associated with an earlier onset; and in fact, many of these babies do not carry the 510C>T polymorphism. So clearly there are other modifier genes or other polymorphisms in the gene. We also know that the monitoring guidance is evolving and may differ based on regions and the variants involved. What I want to point out here today is, in addition to functional studies, it's very difficult to do strength measures. I think looking at the posture of the baby, kinematic concerns are really present and should be evaluated. The frequency of echo and

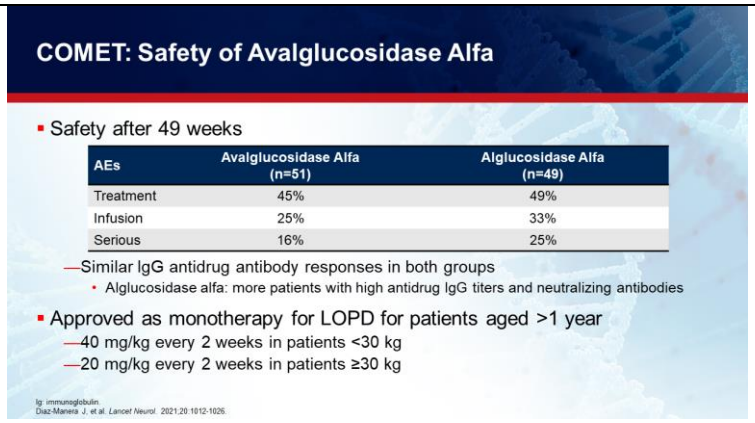
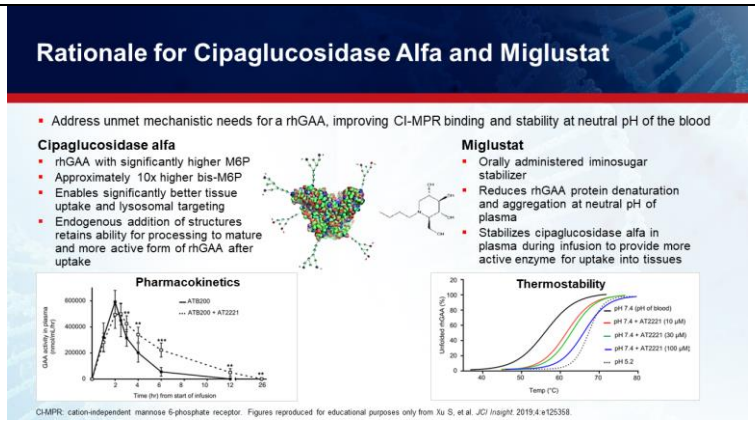
		EKG really should be tailored. If you have the IVS splice site mutation, the frequency of an echo is not much needed. It's cardioprotective. If you have hypertrophic cardiomyopathy in the presence of an IVS pathogenic variant, please think outside the box — this could be due to something else.
32		And so here is beautiful data from my genetic counselor Erin Huggins. This was recently published. This is on 20 babies that were systematically followed at Duke. The vast majority of these had the IVS splice site variant, either in heterozygosity or homozygosity, and I just want to point these pictures to you. What we are finding is a kinematic postural group of features amongst these babies, and there are about 11 frequent findings seen in more than 50% of these patients. What you see is a rounding of the back, a posterior pelvic tilt as these babies sit, a posture of the hips in abduction and external rotation, very tight IT bands, again telling us that their posture is using certain muscles versus others, the presence of scapular winging, which can be quite remarkable. And so I just want us to recognize that there is an emerging phenotype, and I hope that, through time, we will be calling the natural history of late-onset Pompe disease not beginning in the late 20s and 30s, but rather as an earlier onset of disease.
33		

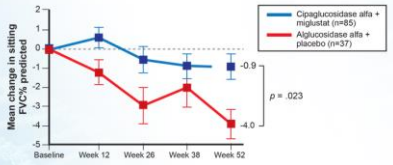
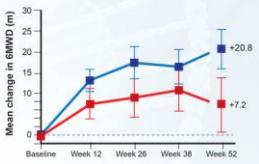
34		And I want to share a video from one of our patients with late-onset Pompe disease, highlighting that there is suboptimal treatment and really the skills… *Good morning. I'm Bob Lee. I'm 84 years old. I was diagnosed with Pompe about 7 years ago.* *New treat therapies.* *It's about 4 years of having the weekly infusions at different sizes. I was continuing to decline physically, I think mentally I was OK, and I began to kind of wonder if I was reaching a point where I was really having to withdraw. I was withdrawing from a lot of activity at this point. I basically started spending more of my time watching TV and I did some reading, but I just didn't have the energy to get up and do things that I wanted to do. So, I had been, at one point, really volunteering a lot, gradually withdrew from volunteering because that required movement from my part and so I just I gave that up. Then with COVID hit, then once essentially things closed down for months. My church was not even open. I was just at one stage, beyond existing.* **Priya Kishnani:** [Now introducing] Doctor Schoser. And what else I wanted the audience to really pay attention to is this patient has very significant lingual weakness and he has a dysarthria as he's speaking. I just wonder if you picked up on that.
35		**Benedikt Schoser:** All right. Good morning to everyone. So, we moved to the next stage. So that was the description of the disease and all our changing knowledge. So, treating rare, is learning rare. That's one of the important things, and that happened to Pompe disease as well.

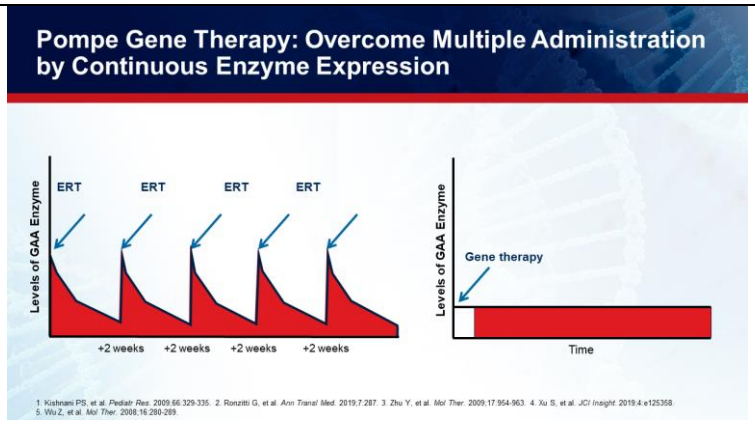
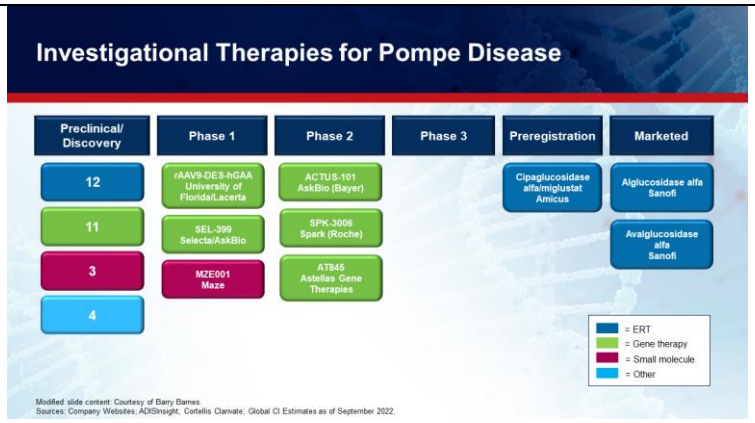
36		Exploring the next-generation treatments, of course, we have different types of strategies currently there in place. We have some replacement therapy. We have new-on-the-block substrate reduction, and of course then the debut of gene therapy, and there are different ways in doing this. And within the next couple of minutes, I'd like to put some points here on this.
37		First of all, what about the enzyme? We have to consider what is the difference between the endogenous and the recombinant enzyme we use for the treatment? And there are some differences, of course. The endogenous is made in the intracellular enzyme, it's made on the cells itself. And we know from the recombinant one that this may escape the mannose 6-phosphate receptor-mediated lysosomal trafficking pathway and also escape and release into the interstitial space. And of course we know pretty well that mannose 6 receptor is a cation-dependent receptor, so energy is needed. So also consider this as well, that's one of the important things. And the final conclusion is, with the current system we have in hand, approximately only 1% of ERT is taken into the skeletal muscle, and there we want to have the enzyme activity. And also there is a decline very easily of this enzyme within the next 24 hours, and we know this as well.
38		So, these are the hurdles and here are already the limitations. So, the impaired delivery is something we still worked on and have to work on, and also the suboptimal uptake. There are several elements we have to consider. And I talked about the receptor-mediated endocytosis and also the uptake via the mono 6-phosphate receptor itself is a limitation, and, therefore, there are some new strategies already explored to overcome this type of situation. But also, we have to consider there's a sink effect. I think that's very important. Substantial amounts of the enzyme from the circulation go into different spaces beyond the skeletal muscle or even the

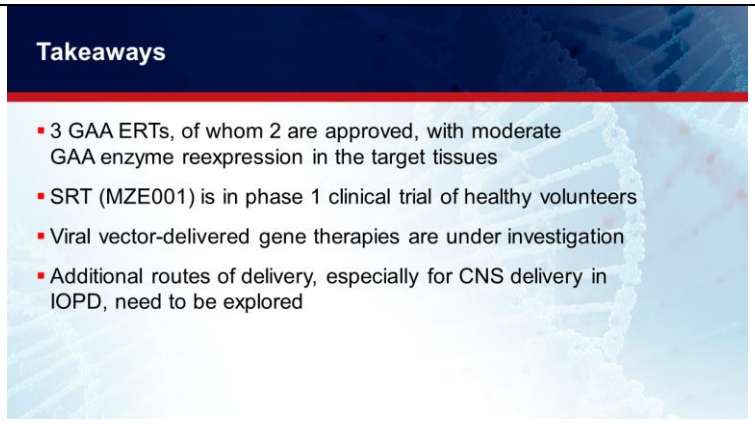
		heart tissue. Also, this is something important: So liver, spleen, and all these organs where normally this type of enzyme is not needed is attracting the enzyme, it's taken up there and also the gastrointestinal tract. But here I still consider GI might be even an important part for the enzyme as well to work. Because you have realized this from Priya's talk that this is a multisystemic disease and not only as we thought in the beginning (LOPD might only be a neuromuscular disease) it's a multisystemic disease and that's very important.
39		So, therefore, here summarizing the ERT limitations factors we have here. Of course, it's the deliverance of the recombinant GAA protein. We have the high volume. The patient needs to take the long hours on infusions. I think that's a burden of treatment we have to discuss, and it's also done every other week to be taken or and the younger ones every week. And the biodistribution is not perfect currently, and on top of this in some even of the LOPD patients, we see the immune system response to the enzyme. Also, this needs to be considered and, first of all, there is something about 1% only taken up, so this is really the summary of this.
40		So, if we look at the next-generation treatments before we do this, what is the disease progression? And here, this is one of the recent summaries we did in our cohort. You see there is a constant, stable decline under the first-generation treatment. So this is clear for all. It's for the motor function, it's for the pulmonary function, and also for the endurance capacity measured by the 6-minute walking test. So, therefore, there's up to a third of individuals that continue to decline. And there might be in some of the patients, even a point of no return. So, if you have a complete muscle wasting or loss of muscle tissue, how should any super enzyme

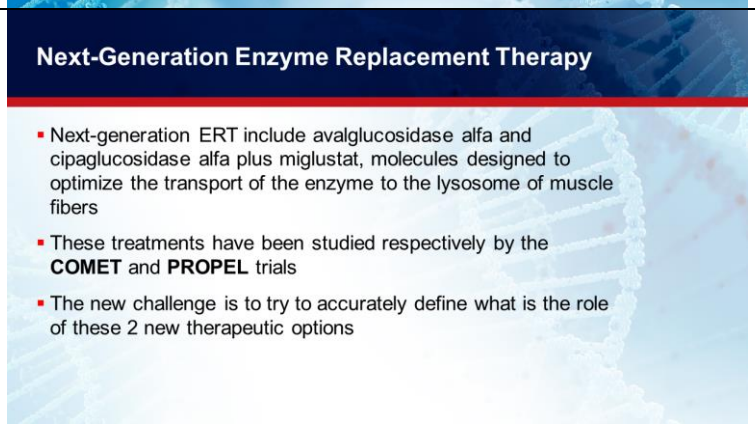
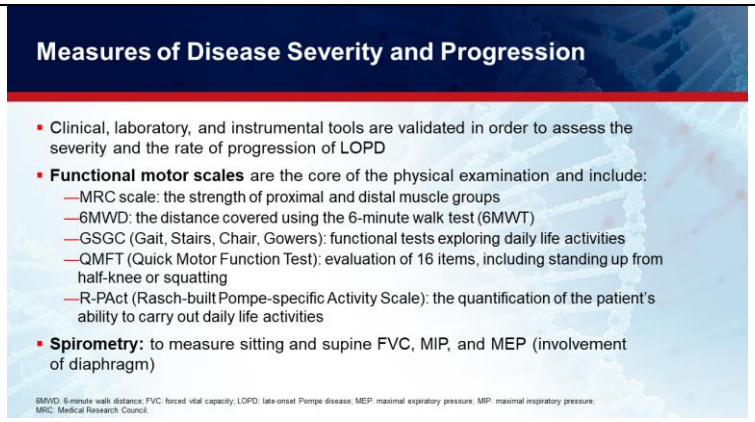
		work there? So, this is something else we have to consider and take into consideration in when to start the therapy in those patients.
41		Looking at the second-generation or next-generation ERT products, of course they overcome some of the hurdles already. So, we know that they have a better,
42		and you see here for one of them, the avalglucosidase alfa, there is more motifs put on so that you have more mannose 6-phosphate receptors on the target and then can deliver it in a much better way. So, this is already a step up from the former enzyme we use still.
43		
44		And if you look at the clinical (so that's the phase 3 data are currently on), and you see here that for the 6-minute walking testing, but also for the forced vital capacity, there is improvement with the next-generation treatment. It's not completely transferring the preclinical data into the clinical reality, but that is a higher ground, honestly. We know this very well that

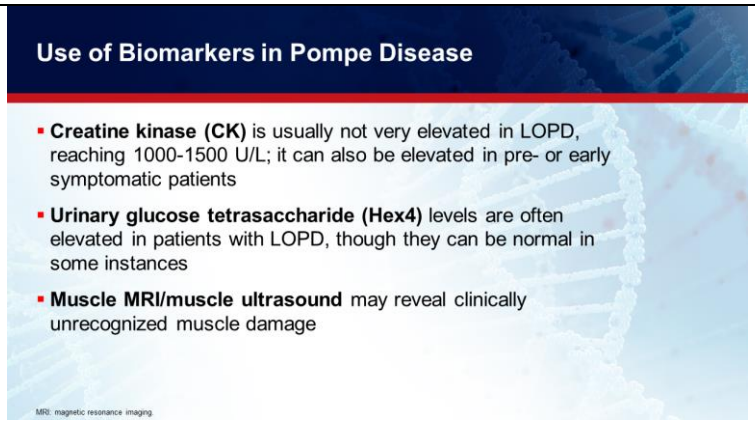
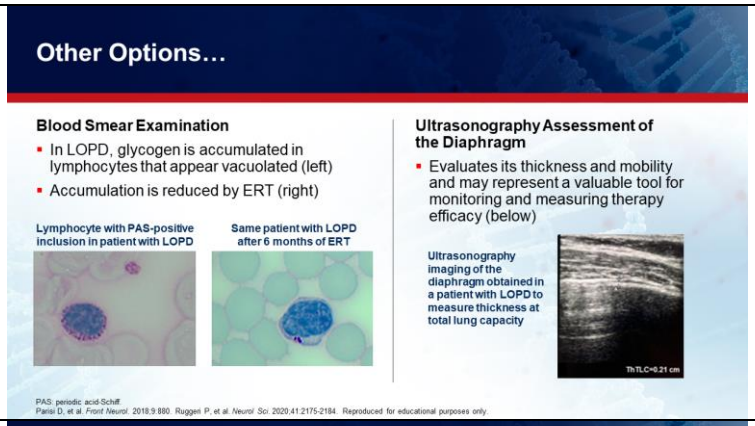
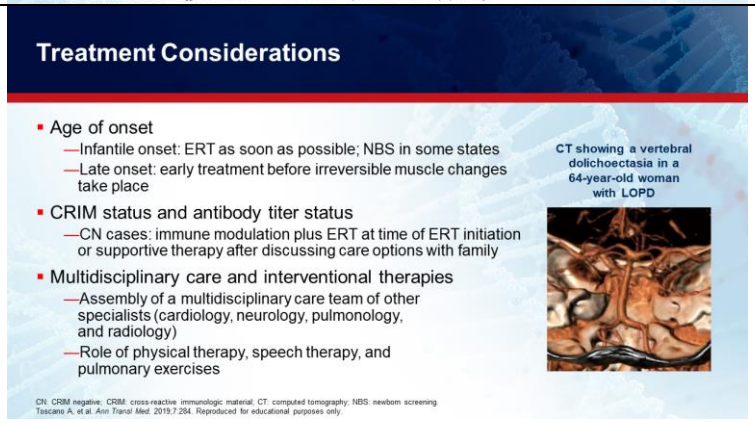
		mice are different to humans, and here, that's the same situation for these new enzymes. But of course it's a step up and it's definitely a step up for both most important functional parameters we still use for our clinical trial readouts.
45		And if you look at the safety, this is something very important. You see similarities and in both cohorts. So, if it's naive, pretreated — untreated and also pretreated cohort — you see very reliable reactions and the pattern is on the mild side. So, therefore, you easily can move a patient from one enzyme to the other; that is definitely given in these safety datas we have already. And this one, the avalglucosidase alfa is now approved over here in the US, but also in many other countries around the world. And also in the label is something else that's new, that you can scale up at least to a certain age and to a certain body weight. And this is something important and we need to consider to do more work on this.
46		And of course, there's a second enzyme. And here you have the combination treatment. It's a different[ly] designed enzyme, having a better intracellular trafficking to the lysosome and doing the work there. And in combination to this, to stabilize this in the bloodstream, we add at the day of infusion miglustat, and this definitely counts on a better pH and also on the thermal stability of the enzyme and getting more active enzyme into the target tissue.

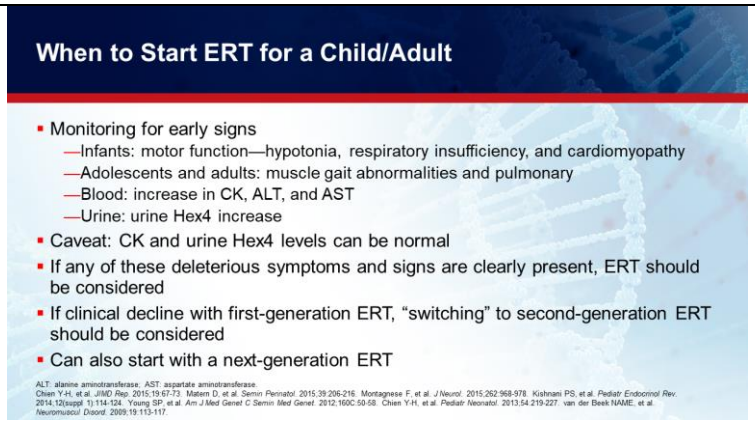
47	**PROPEL: Cipaglucosidase Alfa + Miglustat vs First-Generation ERT—Double-blind, Multicenter, Phase 3 RCT** ▪ Pre-treated and treatment-naive patients with LOPD (N=125), mean age 45-48 years Change in Upright FVC% Predicted  Change in 6MWD  ▪ Cipaglucosidase alfa + miglustat led to improvements in measures of physical and lung function over first-generation ERT ▪ After week 52, the difference between groups in change in sitting FVC% predicted was significant Schoser B, et al. *Lancet Neurol*. 2021;20:1027-1037. Reproduced for educational purposes only.	And this transfers also here, for the phase 3 study, for the PROPEL study, also here in sustained stability and, in part, improvement of the overall cohorts here. We have seen — and this is very interesting findings of course — because you see that the improvement in the long run is also there.
48	**PROPEL: Safety of Cipaglucosidase Alfa + Miglustat** ▪ Safety after 52 weeks AEs: Cipaglucosidase Alfa + Miglustat (n=85) / Alglucosidase Alfa (n=38) Treatment: 95% / 97% Infusion: 25% / 26% Serious: 9% / 3% —2 patients withdrew from cipaglucosidase alfa + miglustat arm due to infusion-associated reactions, one of which was a serious AE —Overall, safety profile of cipaglucosidase alfa + miglustat is similar to alglucosidase alfa Schoser B, et al. *Lancet Neurol*. 2021;20:1027-1037.	So, on the safety side, very similar to what I told you for avalglucosidase alfa, also there is no big difference between pretreated or untreated patients. And compared to the standard of care, you see various similarities and infusion reactions, and also serious events. So, therefore, the overall safety profile also of this second new enzyme in the combination with miglustat is very similar to the former standard of care, and also this is very important.
49	**IOPD: Clinical Trials** ▪ Avalglucosidase alfa —Mini-COMET: phase 2 study in patients previously treated with alglucosidase alfa —Baby-COMET: phase 3 study in ERT-naive patients ▪ Cipaglucosidase alfa + miglustat —Phase 3 study in both ERT-experienced and ERT-naive patients IOPD: infantile-onset Pompe disease. https://clinicaltrials.gov/ct2/show/NCT03019406. https://clinicaltrials.gov/ct2/show/NCT04910776. https://clinicaltrials.gov/ct2/show/NCT04888505.	Going into a different field. So, this was all about LOPD going to IOPD. And here, there's some ongoing situations for the new enzyme. So, for avalglucosidase alfa we have the mini-COMET, that's the phase 2 study in patients previously treated with the standard of care. And we have the baby-COMET, that's a phase 3 study in naive patients. And on the other hand, we have the ongoing phase 3 study with ERT-experienced and -naive patients for the cipaglucosidase alfa.
50	**Mini-COMET: Avalglucosidase Alfa Stabilizes or Improves Mobility and Motor Function in IOPD** ▪ Most participants 18/22 (82%) had motor decline at enrolment, others suboptimal response ▪ On avalglucosidase alfa —Motor function was stable or improved for all 4 groups —Pompe-PEDI Functional Skills Scale—Mobility Domain was stable or improved **Change From Baseline at Week 97** Cohort 1 AVAL 20 mg/kg QOW: GMFM-88, total % score, mean (SD) +5.05 (17.50) [n=6]; QMFT, total score, mean (SD) +0.50 (6.89) [n=6]; Pompe-PEDI Functional Skills Scale—Mobility Domain, mean (SD) +6.71 (12.66) [n=6] Cohort 2 AVAL 40 mg/kg QOW: +0.94 (1.33) [n=3]; +2.33 (8.74) [n=3]; +3.01 (2.30) [n=3] Cohort 3 AVAL/AVAL 40 mg/kg QOW: +4.32 (10.79) [n=4]; +4.00 (6.48) [n=4]; +4.61 (2.77) [n=3] Cohort 3 ALGLU/AVAL: +9.85 (7.57) [n=6]; +7.17 (7.36) [n=6]; +10.94 (6.35) [n=6] GMFM-88: Gross Motor Function Measure-88 items; Pompe-PEDI: Pompe-specific Pediatric Evaluation of Disability Inventory; QMFT: Quick Motor Function Test; QOW: every other week. Kronn D. 18th Annual World Symposium. Abstract 156.	Here's some results from the mini-COMET. You see that the avalglucosidase alfa stabilizes or improves the mobility and motor function in a lot of scores here. Just having the GMFM-88 score, the total score, and you see there were different cohorts with different enzyme levels and different dosages used, and you'll see an improvement in all of these cohorts. That's very

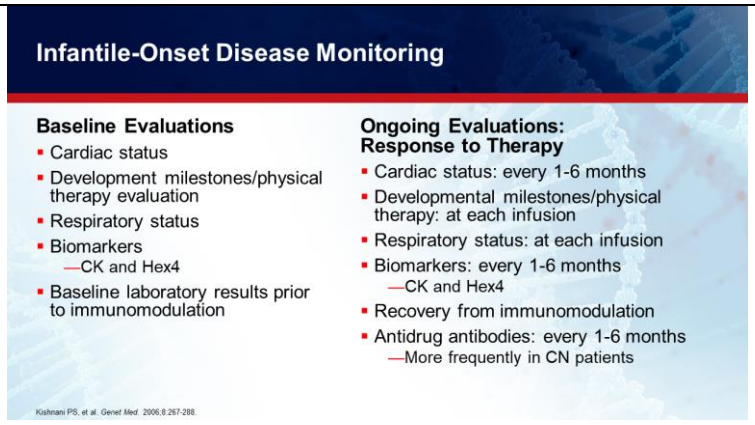
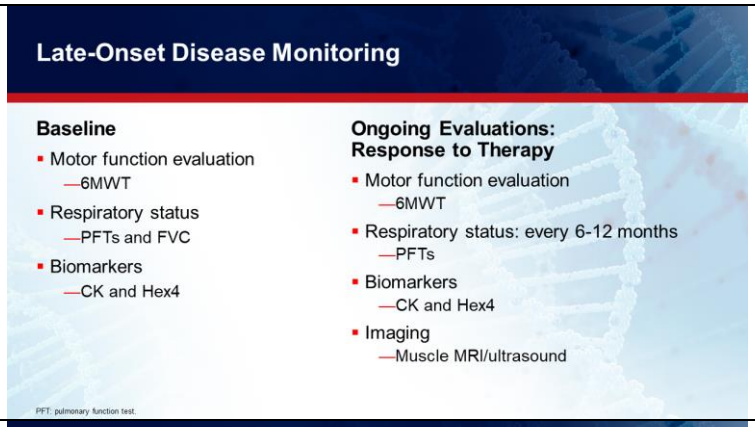
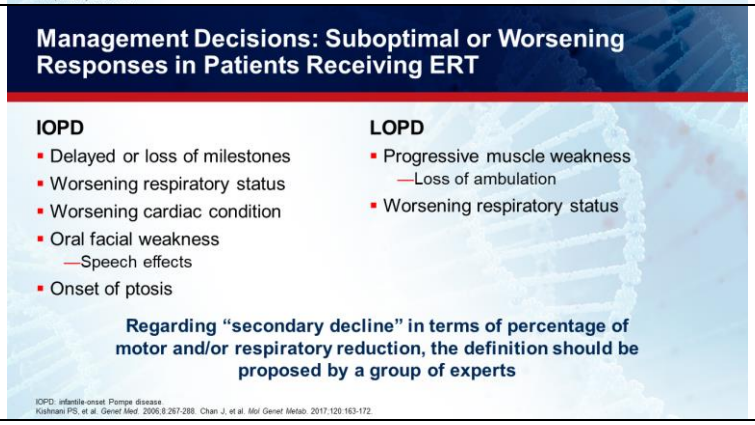
		important. And also from the quick motor function test, you see the total score is improving in the cohorts. And also the PEDI, the Pompe-PEDI, a very clear functional skill scale for mobility in the babies is also a move forward in the right direction. So these are really good news, that it also works in the IOPD population and therefore the label is correct for these enzymes. That's very good news.
51		But, I was already talking about the disease and the treatment burden here and ERT treatment burden is a high one. You have to come as a young adult every week or doing at home; that's the alternative, of course, in some of the countries, and having your infusion. And this, well, in a way, steals away about 2 to 4 to 6 hours of your life every week, or at least every other week. And this is, for the adults, also a big issue. So, therefore, moving on from ERT to something like a gene therapy where you have a prolonged sustained enzyme activity like we all have here in the room. So, this is our situation — going into a normalization of the treatment; that would be a very nice occurring.
52		And therefore we have a landscape now, and let's just glimpse here, I don't go into details here. We have a landscape of additional issues here with the gene therapy. There are different ways in doing this. There's AAV vector-based systems with also antiviral-based systems in use and also, on top of this, we have something else. So, we have the substrate-reduction very early data pointing in the direction that also this might be a very helpful way. So, therefore, the full-glance picture currently is really having established ERTs — first- and second-generations — and going into gene therapy. And this will be like a puzzle. So, each and every patient might be a different one and it's really precision medicine. Needs to be done here to have the right way, the right treatment, at the right time point, and

		for some of them there might be a very specific time point. Gene therapy won't work in all patients. It will work in some of them at a very specific time window. As we know from other diseases, that not all fix everything to every time point, and this will happen here again and, similar, we will not get rid of ERT in the long run. So we will also need ERT, and even have then a combination treatment of substrate reduction, of gene therapy and ERT — different types at different time points — and have here now, for the first time, the situation that we can have a shared decision also with the patients: How to continue? What to do? and What is the best for you to overcome the situation? And the next step, of course, will be: Can we prevent the disease? So going in the early stage of the disease, especially in LOPD (in the IOPD there the situation is a bit different), there we need additional treatment, especially on gene therapy. We need something that is tackling the central nervous system. But for the late onset, it might be that we have to move our mind. So I was always on a different side, but I have to move my mind myself. Getting in early treatment and trying to prevent the disease, and I think that's a very important one.
53		So, the takeaways: We have three different ERTs, two of them are approved over here. And they are moderate in the re-expression in the target tissue. We have the substrate reduction in the early phase 1 clinical trials. We have different types of viral vector-based delivery systems in place and in clinical trials, and, of course, we need something else on top for our infants to treat them best. Thank you.

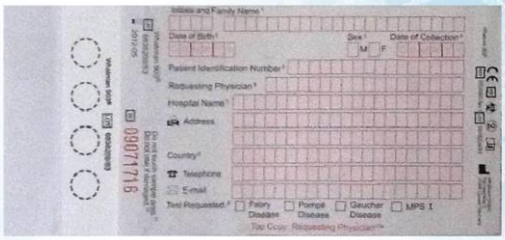
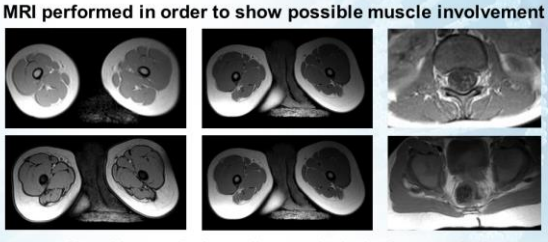
54		**Antonio Toscano:** Good morning, ladies and gentlemen. I, first, would like to thank the organizer of this congress, and especially the organizer of this exciting symposium, our course director and my friend Benedikt Schoser to have involved me.
55		So this is something, this talk is some medium discussion because we need to take together all the results that Priya and Benedikt have already shown that are very important, especially in the view of new treatments, new important weapon to fight the Pompe disease. So this new-generation ERT are relevant, we really believe in them, and we have an important role to establish how to use them. And this is not easy because we still don't know long-term results and we have to be careful to apply these new therapies.
56		So, you all know that there are several possibilities to diagnose and follow the patients, either from the motor point of view or the respiratory point of view. And this list you see is the list usually working with these patients. And you see that the MRC scale, 6-minute walking test, the temporized tests, as for example GSGC, the QMF test for all the evaluation of motor functions and the R-PAct — which is important because it can show you the results of daily activities of patients. But we cannot ignore, of course, the study of respiratory parameters, especially by spirometry, using this for establish the results from FVC supine or sitting, or MIP, MEP, and other possible parameters. So, this is important, because you know that the most affected muscle for respiratory

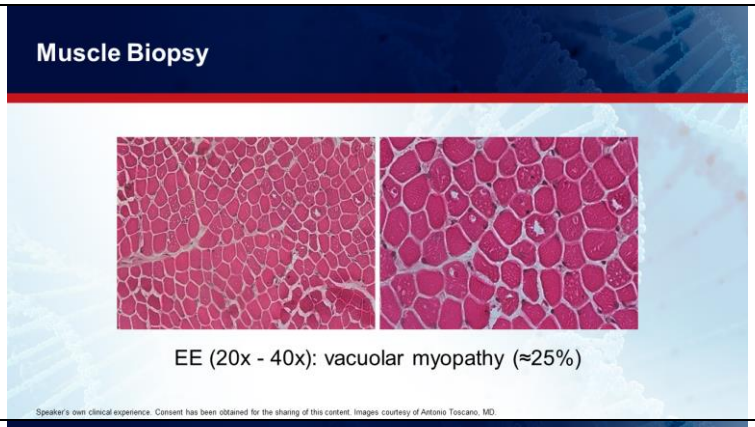
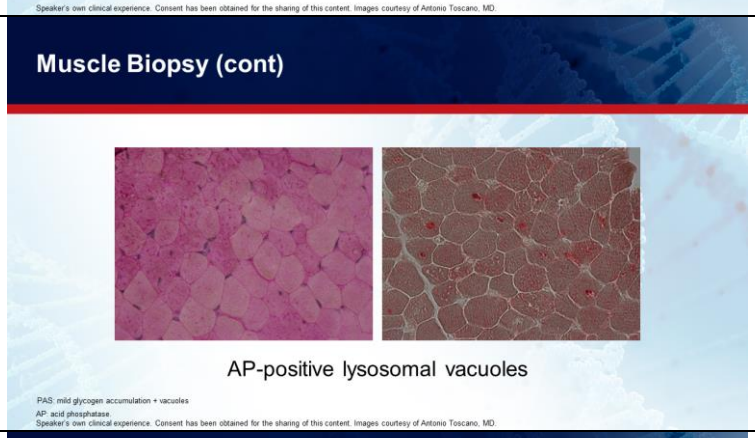
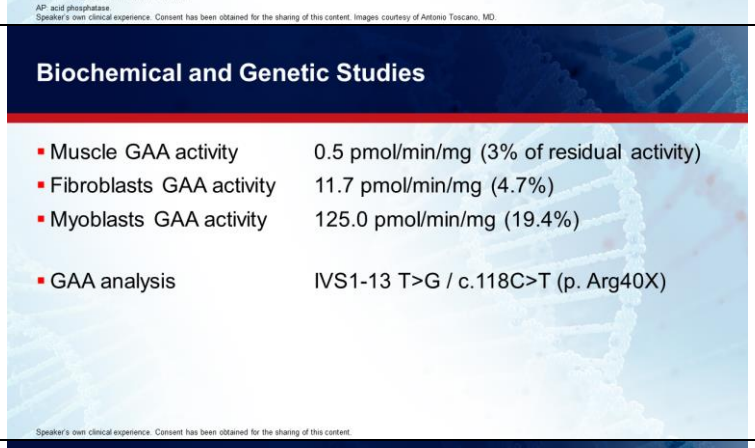
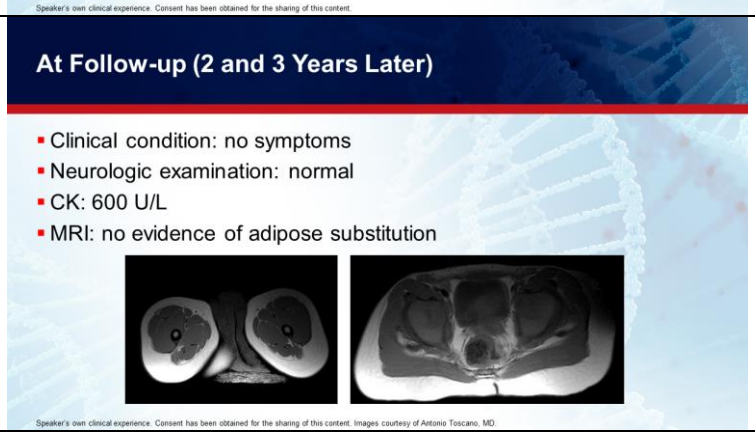
		purposes is the diaphragm, so we can test the diaphragm.
57		And now, of course, also the laboratory can help us, partially, I would say, because we can have the possibility to look at the levels of CK or transaminases. And you know that, whereas in the infantile cases the CK is a little bit higher, reaching even over 2000 units per liter; but in the LOPD patients, the level is not farther than 1500 units, and this can be also found in early or presymptomatic patients, sometimes. So urinary glucose tetrasaccharides, commonly called Hex4, is also an important test to establish if there is a normal excretion of this metabolite. But even more important, in my opinion, is the use of muscle MRI, which is very diffuse now. It is very important because it can allow us to detect some early muscle degeneration that can escape the clinical examination.
58		
59		As Priya and Benedikt pointed out before, we have to consider several other points to take in mind: how to start and when to start and what kind of therapy we have to use. One point is, of course, the age of onset, and you know that there are two forms, the infantile and late onset. The CRIM status, which is more relevant for infantile cases but not really for the adult cases. And we have to take in again into account, that because of the multisystem disorder, which is clearly recognized now for Pompe disease, we have to take in mind that we have to check these patients for heart, for GI tracts, for urinary aspects, hearing, and vascular

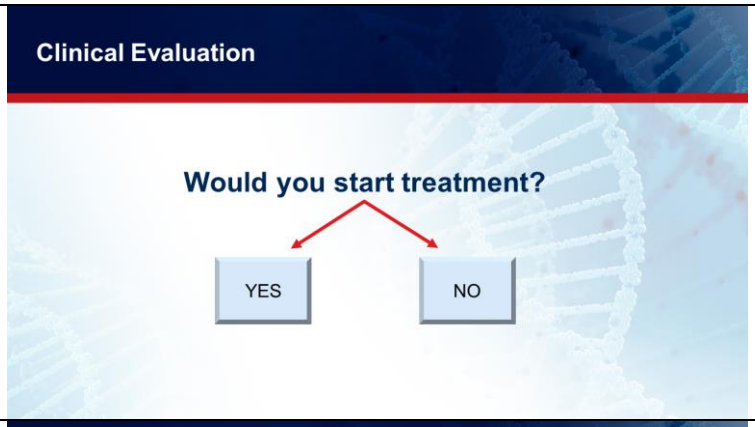
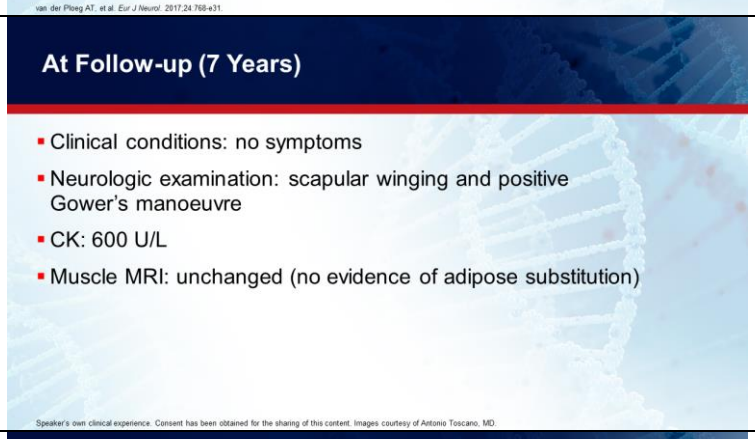
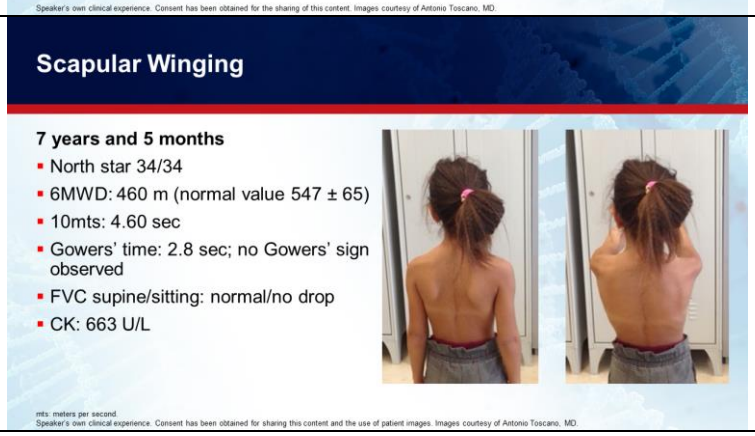
		problems. And this is very important, because we need to involve other professionals. And you'll see that the list of professionals increased a lot in the last 10 years because of the multisystem involvement. Don't forget that apart from trying to choose which is the best therapy for the patient, assuming that now we have more possibility to treat patients, we have not to neglect the possibility of the rehabilitation therapy, which particularly involves the speech therapists, the physiatrists, and the physical therapists. So, these are very important, I would say, complex condition. You see on the right-hand side, for example, is a case where a patient which was not treated, for her decision, for several years. She was hypertensive. When she came to us, we visited her and we had the neuroimaging and we found a very big aneurysm, but that was eliminated suddenly because she was at risk. And after that she decided to start therapy.
60		Trying to summarize. For infants and adults, it's important to understand what is the real motor situation, the respiratory situation, especially for infants, the heart situation, which is very important. We use laboratory, laboratory examinations. Don't forget that in some cases CK and Hex4 can be normal. So, taking all this information in mind, the new aspects we have to discuss are especially when we have to start ERT. So, this is a very open discussion because we have some rules, and I'll go back to this in a few minutes, but things changed. For example, the use of MRI disclosed other aspects of the muscle degeneration. The second point is, when we have to switch from one ERT to another. So, this is another point, and we need longer studies to have more precise data. And finally, of course, now we have another option to start therapy with ERT according to the results of the recent trials.

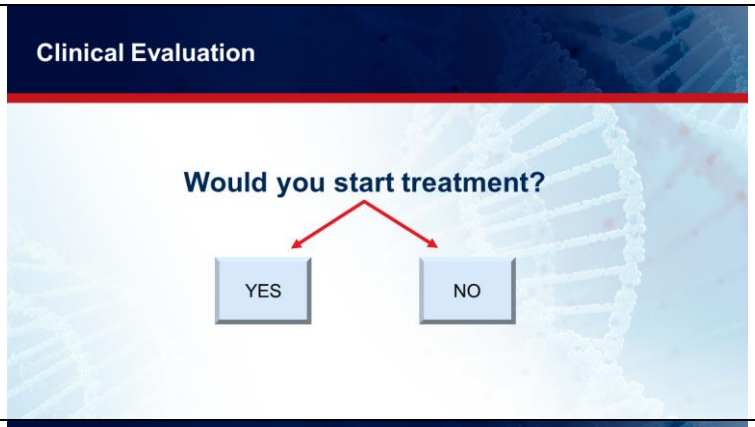
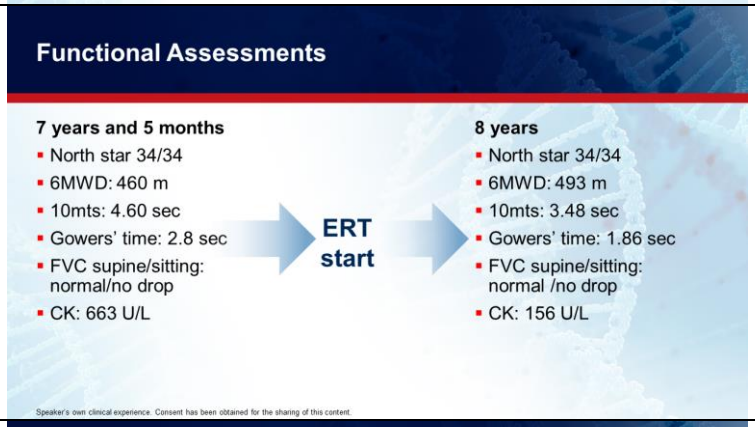
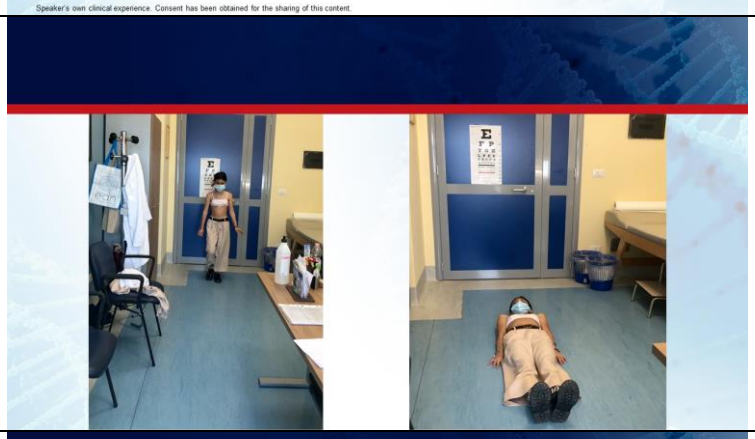
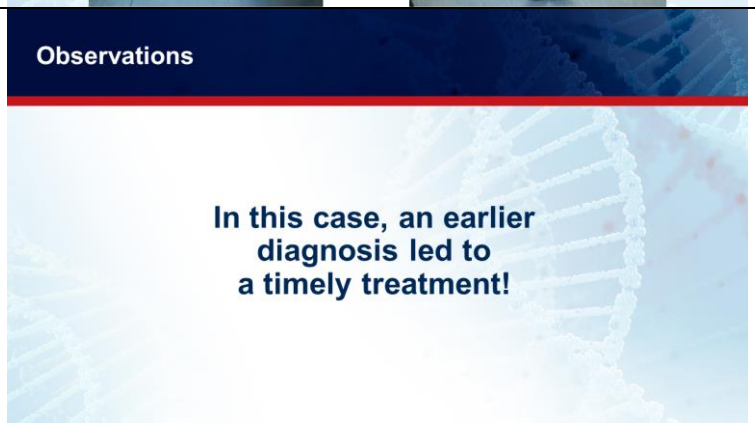
61		Priya has already described largely the infantile form; I don't need to go deep in this discussion. Don't forget that the cardio status is fundamental to be examined; but also, of course, we have to follow the milestone, the motor and respiratory milestone of the babies, and the biomarkers also can help. But of course, the final decision for the diagnosis is the molecular genetic study, and this is very important to be performed in timely, because we can start very soon the therapy when needed. And we have to evaluate the patient, and the laboratory examination and clinical examination are similar — either when you have to establish the diagnosis, but also to follow the patient. It's important to outline that, for infantile cases, it's really relevant to check the antibodies level, because the antibodies level can influence the result of the therapy.
62		It's similar situation in the LOPD patients. You have to check motor function, respiratory function, imaging as regard as muscle MRI and biomarkers. And this is also the list of things we have to perform when you have to check the patient every 6 or 12 months.
63		

64	**Patient-Reported Outcome Measures** ▪ In addition to the clinical results, a **subjective quality of life assessment by patients should be taken into account** ▪ Validated patient-reported outcome (PRO) instruments may include: —PROs Measurement Information System (PROMIS) —Pompe Disease Severity Scale (PDSS) —Pompe Disease Impact Scale (PDIS) —R-PACT —General PRO • 12-Item Short-Form Health Survey (SF-12) • EuroQol-5-Dimension-5-Level (EQ-5D-5L) • Patient Global Impression of Change (PGIC)	But, this is another important, let's say, recent acquisition because we need to know and we need to hear the voice of the patients. And this is the result of what has been done recently. So, several validated patient-reported outcomes have been applied to the recent trials and you see that there are several scales, new scales, but also some already used scales. Like, for example SF-12, that are very important to disclose to us the real importance this patient gives to the therapy and the progression of the disease.
65	**Multidisciplinary Care Team**  	And this is something that, in summary, show you how large is the number of professionals included in this, in the care of Pompe disease.
66	**Clinical Case Challenge**	So, to finish, I would like to show you a case.
67	**Clinical Presentation** ▪ 2-year-old girl, born from non-consanguineous parents ▪ Family history negative for neuromuscular disorders ▪ Normal developmental milestones ▪ At 1 year of age, she presented with a laryngeal inflammation ▪ A routine blood examination was performed; mildly elevated serum CK level was present (519 U/L, normal value 0-200 U/L) ▪ After 3 months, her CK remained moderately elevated (600 U/L) Speaker's own clinical experience. Consent has been obtained for the sharing of this content.	This is a 2-year-old girl. No history of neuromuscular disorders. Because of a laryngeal inflammation at 1 year she had mildly elevated CK, 600 something. This was repeated after 3 months.

68	**What Did We Find?** ▪ Neurologic examination: normal gait, able to run; no evidence of muscle weakness ▪ Electrocardiogram + echocardiogram: normal ▪ Respiratory evaluation: normal ▪ Blood test: hyperCKemia (598 U/L) Speaker's own clinical experience. Consent has been obtained for the sharing of this content.	When we saw her clinically, she was unremarkable, no neurologic or cardiac or respiratory problems. Still, CK elevated, mildly elevated.
69	**DBS for GAA Activity** GAA activity: MARKEDLY REDUCED (0.39 µmol/h/L, normal value 1.86-21.9 µmol/h/L)  DBS: dried blood spot; GAA: acid alpha-glucosidase. Speaker's own clinical experience. Consent has been obtained for the sharing of this content. Image courtesy of Antonio Toscano, MD.	And we performed the DBS, and DBS gave us a very low result.
70	**Diagnostic Evaluation** How to support the clinical suspicion of Pompe disease after the DBS result? ▪ Muscle MRI ▪ Muscle biopsy ▪ Biochemical studies ▪ Genetic testing Speaker's own clinical experience.	Because of this, we applied the usual diagnostic techniques, starting from muscle MRI. But we also perform other studies. I would like to outline that, in some cases, especially infantile cases, of course, the genetic testing precedes all the other possible studies to get as soon as possible the result of the diagnosis.
71	**MRI Features** **MRI performed in order to show possible muscle involvement**  No evidence of sclero-adipose substitution in paraspinal, iliopsoas, and posterior thigh muscles Speaker's own clinical experience. Consent has been obtained for the sharing of this content. Images courtesy of Antonio Toscano, MD.	This is the result of MRI, which was unremarkable.

72		But the muscle biopsy showed that over 25% of fibers were already degenerated and maculated,
73		confirmed by stain for PAS and acid phosphatase.
74		But what was really surprising was the residual activity that was 3% — very, very low. Fibroblast and myoblast also altered, and the confirmation of the diagnosis came from the *GAA* analysis, where there was the common change IVS-1 plus a second change.
75		We followed the patient for several years after that diagnosis, and still she was stable with the CK mildly elevated.

76		
77		And we were asking us, we have to start treatment, but according to the European Pompe Disease Consortium, at that time, 2017, we assessed that the patient should be symptomatic to start the therapy. And in that case the girl, the little girl was not symptomatic.
78		Going ahead and checking her every year, when she was 7 years old, she started to show problems in Gower's maneuver, which was not exactly well done. And the muscle MRI was unchanged, but still the CK was high.
79		But if you look at this, and this similar image to what Priya showed, you see that there is scapular winging. Also she had a quite important, still important elevation of CK. But also the 6-minute walking test was a little bit less than the usual limits.

80		In this case, with this clinical evolution, we decided to start the treatment.
81		And when she was 7 years and 5 months, these were the results. If you look 6 months later, the 6-minute walking test was within the normal limits. The time for the temporized Gower's test was improved and CK was really normal.
82		This is the girl now; she's 11. It seems that the therapy has worked very well.
83		And what this case teaches us — so when we have the opportunity to have an early diagnosis, we can start at a timely treatment. So, this is important for all of us. This is to finish, and I would like to remind you that there are still open questions about what is the consideration of decline in these patients, because we know that, as Benedikt alluded, there is a decline of the performances either from the motor, or from the respiratory point of view and we should better define this. And also, now we have the opportunity to use

		different approaches from the therapeutic point of view and to start with a different approach as regarded as the past. And this is, that's all. Thanks very much for your attention.