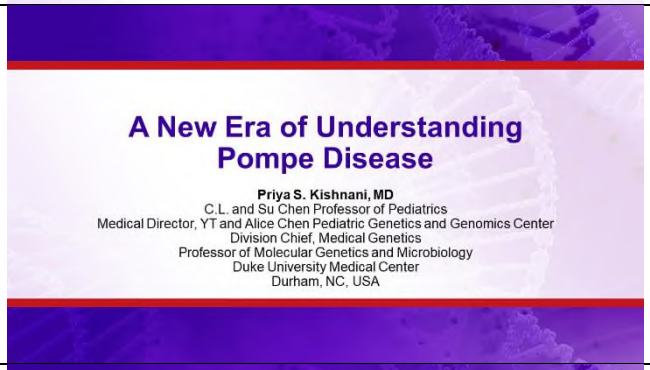
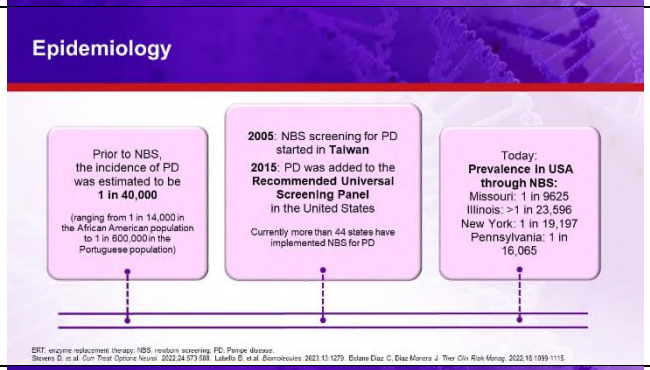
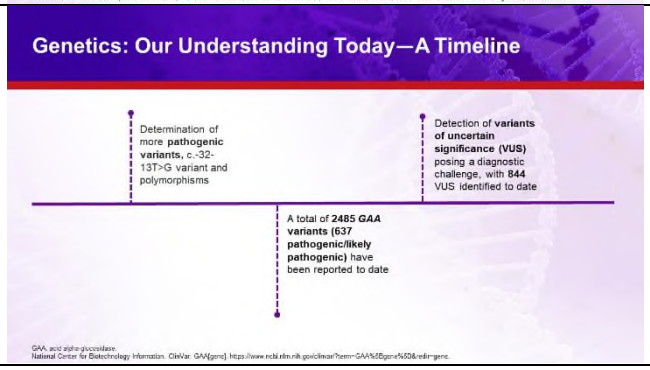
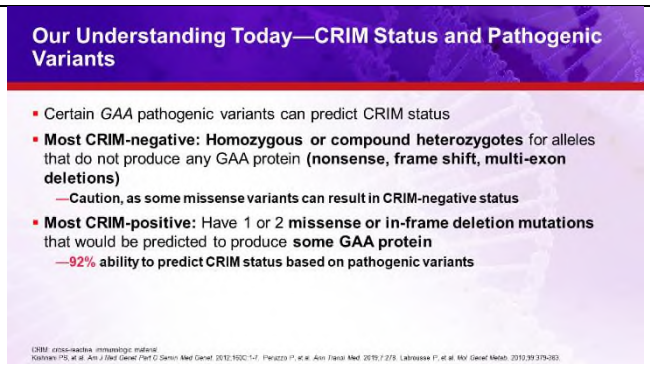
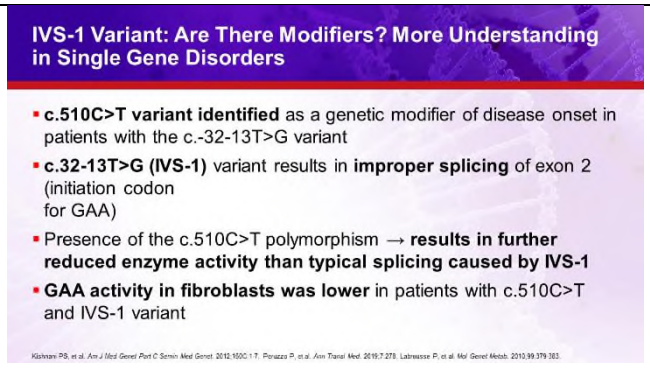
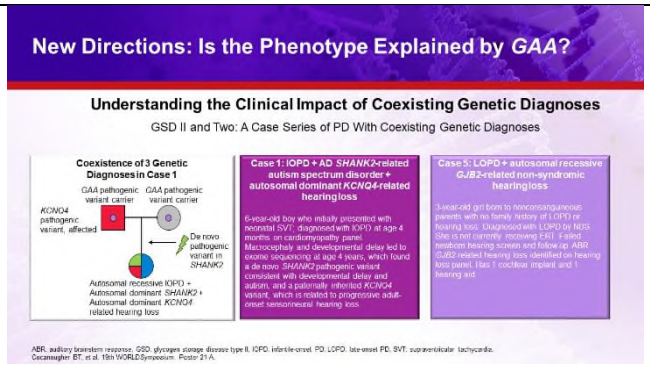
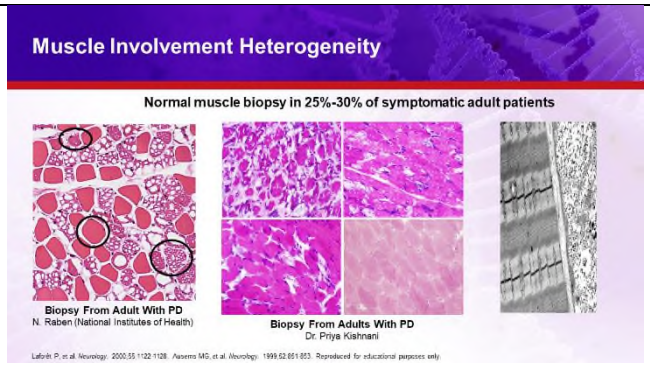
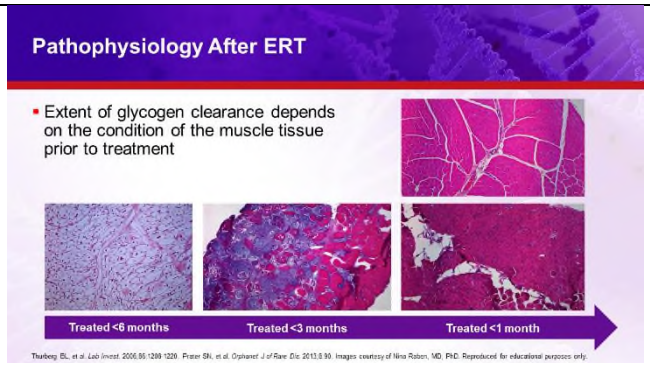
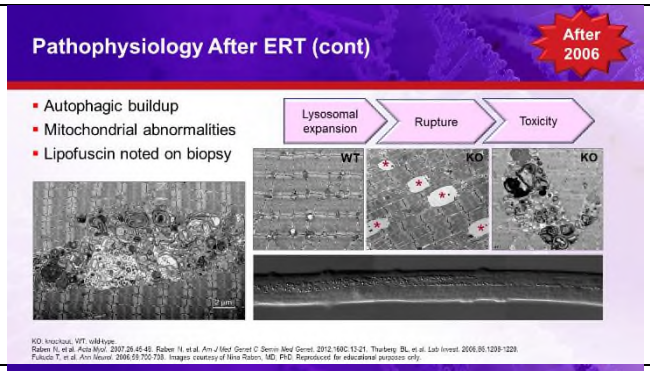
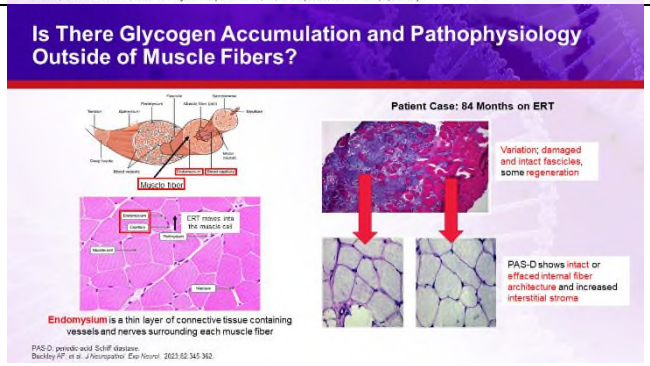
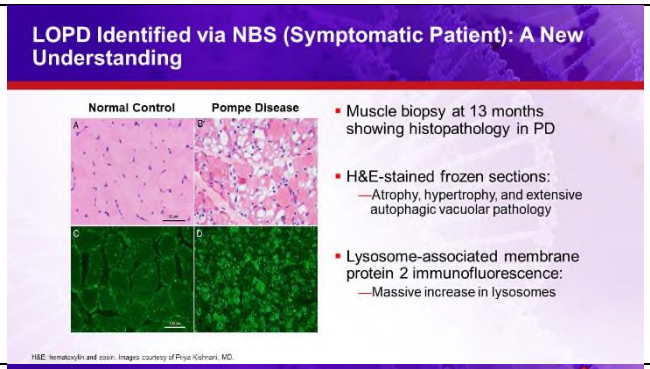
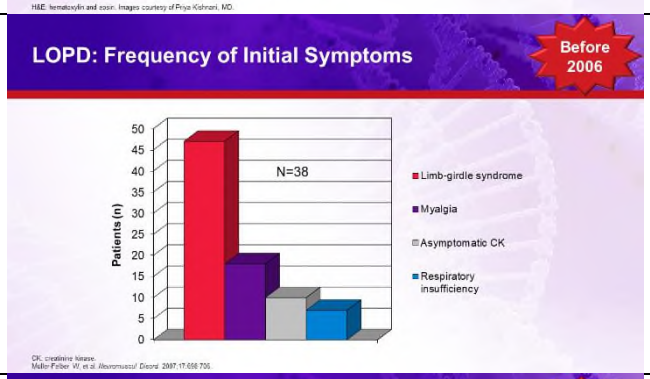
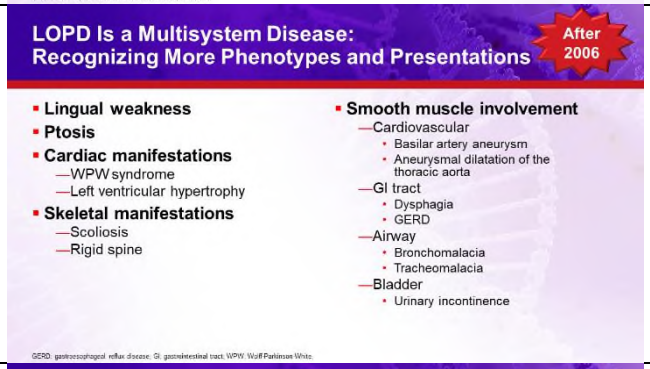
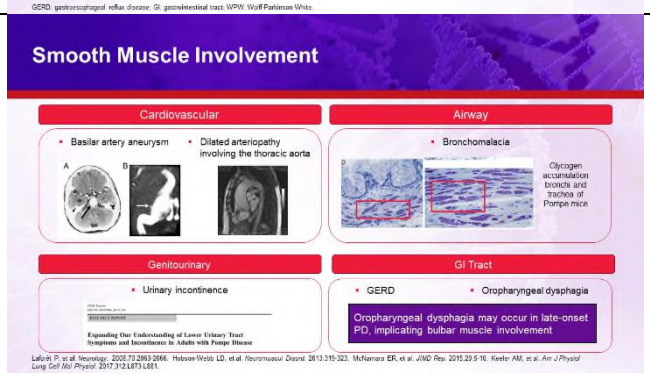
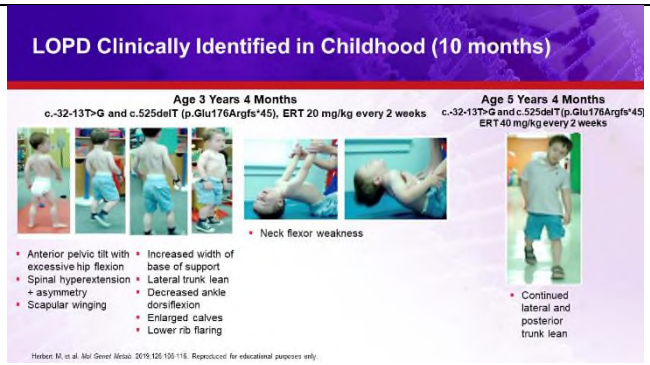
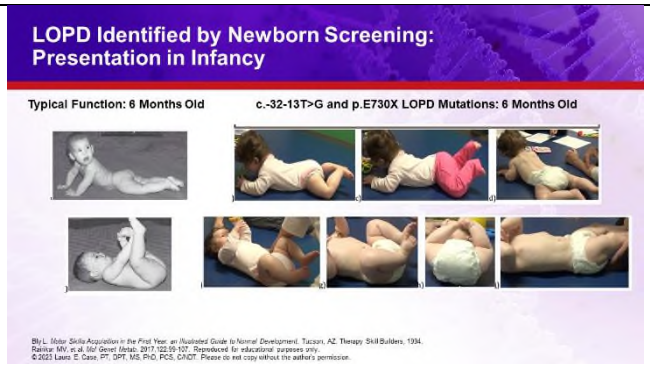
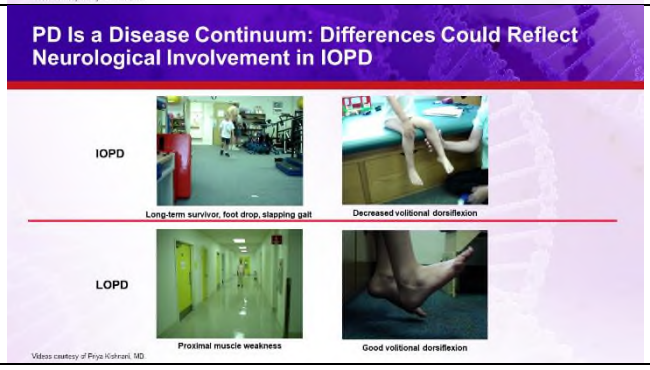
Optimizing Outcomes in Late-Onset Pompe Disease:
Integrating New Therapies, Whole-Person Markers for Disease Monitoring, and Shared Decision-making Into Practice

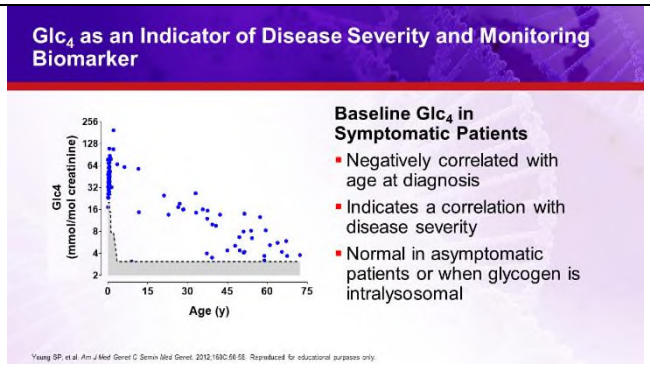
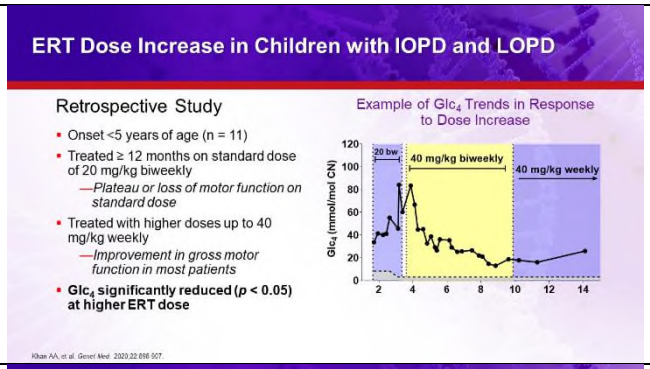
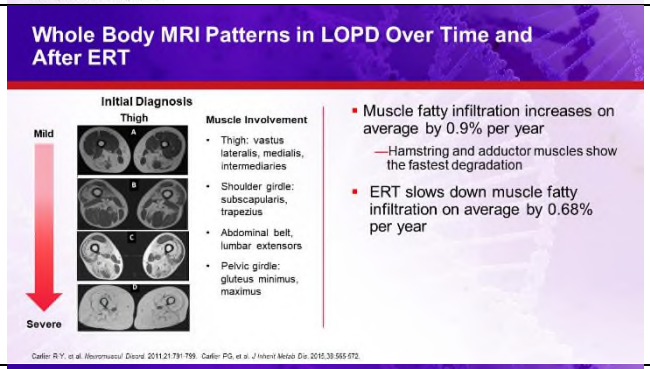
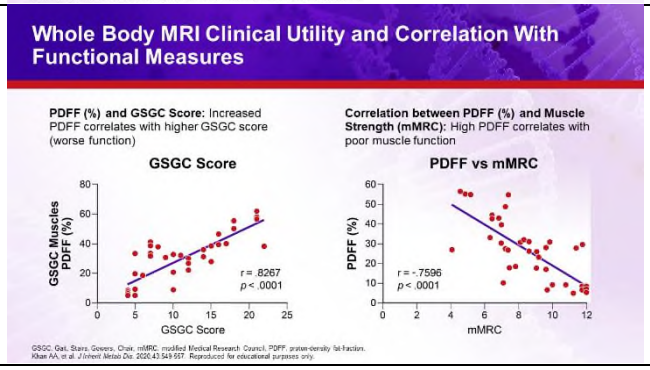
1.		Good morning everyone, and thank you for coming to this very early morning symposium on trying to enhance our understanding of late-onset Pompe disease.
2.		And I'm really privileged to have Dr. Mark Roberts. He's a neuromuscular physician and heads the unit in UK. And then we have Dr. Benedikt Schoser, who's also a neuromuscular physician and is in Munich. So you can see this is a very international group here.
3.		And let me get started.
4.		So I think the first excitement is our understanding of epidemiology. The textbooks have clearly stated that the frequency is about 1 in 40,000. Newborn screening has clearly taught us that the frequency is almost twice as much, about 1 in 18,000 to 1 in 20,000, both from studies in Taiwan and in the US.
5.		In terms of variants, in 2006 we knew of about 120 pathogenic variants. Today we have many more. We also know that the common leaky IVS splice-site mutation is very, very common amongst LOPD, especially amongst Caucasian patients. If you look at ClinVar today, there are close to 2500 variants. But if you also look at ClinVar, there are about 900 or 850 variants of uncertain significance, which clearly poses a challenge in itself.

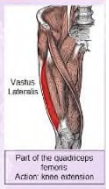
6.		Now let's talk about CRIM, an old story which we used to do by western blot. Today we can do it by mutation analysis. It's very clear that you can look at databases, and usually patients who are CRIM-negative or those who have absolutely no residual enzyme have a combination of nonsense, frame shift, multi-exon deletions. But a caution, there could be some with missense variants, and thus really consulting with your molecular team and looking at databases is very important when assigning CRIM status. Today we can predict about more than 90% of patients based on known pathogenic variants. But here's again the caveat. The VUS situation often comes in.
7.		Let's go back to the IVS variant. Why is it that patients can present anywhere from the first year to up to the 6th decade? There clearly are modifiers within the gene as well, and we know of one, which is the c.510C>T variant, and this is now identified as a modifier. When it's seen along with the IVS splice-site variant, what it does is that it results in a further reduction in enzyme activity than typical splicing, which would be caused by the IVS-1. So in patients who have early-onset, late-onset Pompe disease, they could carry the c.510C>T. However, in the US newborn screening cohort we've hardly seen but 1, and still patients presenting earlier. So there's much more that is to be learned over time.
8.		The next one is, can we really explain a phenotype fully by *GAA* variants? I think we all have to think outside the box. When you start seeing a clinical phenotype that's not completely consistent, or if you see very early-onset of sensory neural hearing loss, as an example, these are just some cases that we have identified now in our Cohort at Duke, which have labeled GSD II and Two, and sometimes it's GSD II and Three. So you can have *SHANK*2 mutations when you have someone with the autism spectrum disorder or someone with hearing loss, and you can have *GJB*2 variants. And so more to be learned in single gene disorders.

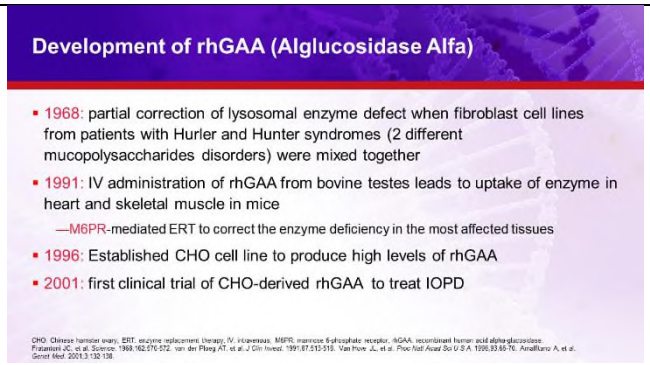
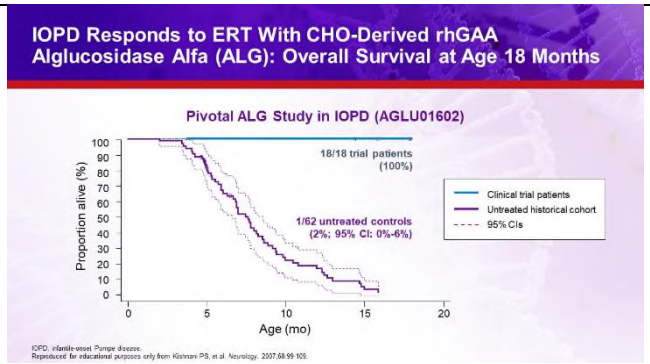
9.		Now, let's talk about muscle involvement heterogeneity. Again, our focus here is late-onset. So if you look at the literature, about 25% to 30% of symptomatic adult cases can have a normal muscle biopsy. Again, recall where this biopsy was taken. But if you see to the left, there's a lot of heterogeneity within the individual muscle fibers, and then you can have completely normal muscle, which is to the bottom right. And then if you look at EM, this fiber variability is even seen at the level of EM, completely normal, surrounded by completely involved muscle fiber.
10.		So I think we've learned a lot in the space of enzyme therapy, what you and where you start is where you end up. So if someone has started at just under 6 months of age, you can see that after enzyme therapy, there's still significant glycogen accumulation in this quadriceps muscle biopsy. If someone is treated at less than 3 months of age, this is the case with infantile Pompe. So these are just learning examples. You can almost see a dimorphic response with some very healthy looking muscles and some very involved muscle fibers. And then when you start at less than 1 month—which is really the goal of newborn screening—you can see very clean muscle fibers after the initiation of enzyme therapy. So this is one story that we've learned.
11.		Then, through brilliant work from Nina Raben, we've learned the role of defective autophagy and autophagic buildup, which can also occur right in the newborn screening setting with muscle biopsies for patients with LOPD telling us that the disease pathology is starting early, we know that there's downstream mitochondrial involvement and abnormalities.
12.		And now what we are also learning is, is there glycogen buildup and a pathophysiology occurring outside of the muscle fiber. And by this, I mean the endomysium, which is that thin layer of connective tissue containing vessels and nerves. And the key here is blood vessels surrounding each muscle fiber. Remember that we are dependent on enzyme therapy delivery through these capillaries and blood vessels. And so now, if you look to the right, what you're going to see is very healthy looking muscle. And there, there's very good glycogen clearance.

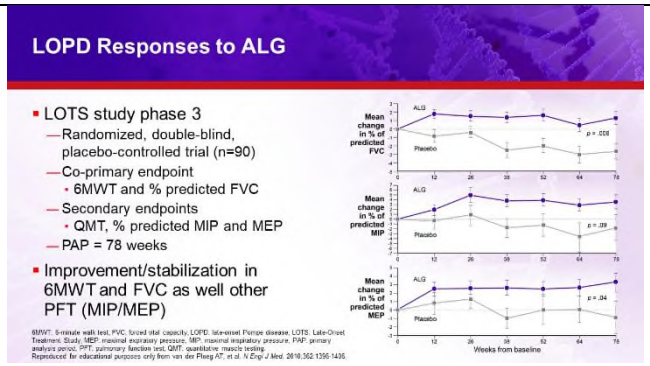
		And now when you look at the other, you can see here that there is buildup of some material in the interstitium. And I think this becomes another impediment to the delivery and also to the response of enzyme therapy.
13.		All right, now let's look at newborn screening. A patient with late-onset Pompe disease and the IVS splice-site mutation to the right is a baby at 13 months, already 2 months on enzyme therapy, and you can see the amount of vacuolar buildup and glycogen accumulation in this particular baby. So, once again, got to think and manage very clearly.
14.		Now, let's talk about initial symptoms. This was our understanding before 2006, which was when AVAL glucosidase-alpha was approved. We thought of it as a limb-girdle muscle disease, approximate limb-girdle disease.
15.		Today, we've clearly understood that it is a multisystemic disease with more phenotypes and ways a patient can present, including with dysarthria and tongue weakness, eyelid ptosis, cardiac manifestations like WPW, and skeletal manifestations. But I also want to focus on smooth muscle involvement because this has become a new area of learning.
16.		So you see this in the cardiovascular system in terms of the ascending aorta and other blood vessels, where you can have a dilated arteriopathy. You can see it now in the airways in terms of bronchomalacia. You can see it in the genital urinary system, in terms of urinary incontinence and anal sphincter incontinence. You can also see it in the GI tract. Many of our patients do talk about oropharyngeal dysphagia, but also in terms of other GI challenges, such as frequency of diarrhea and alternating constipation.

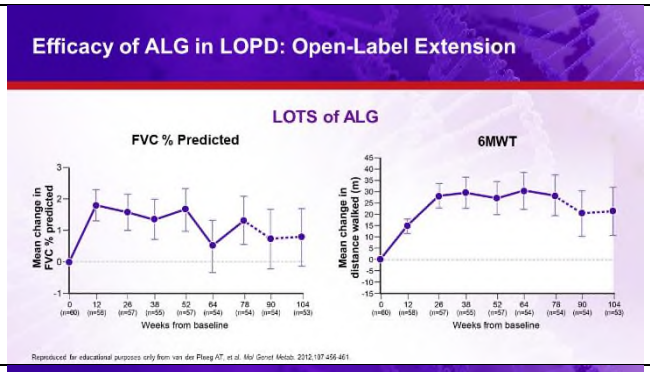
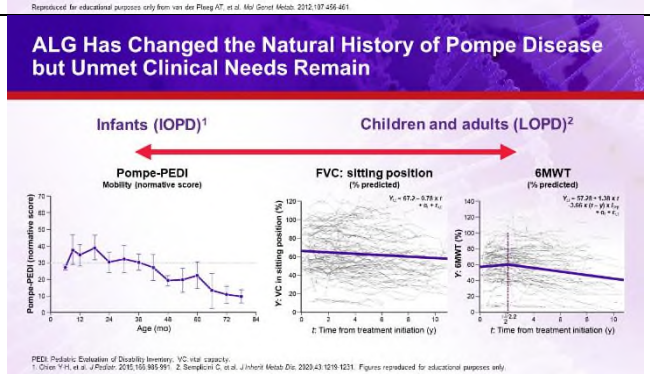
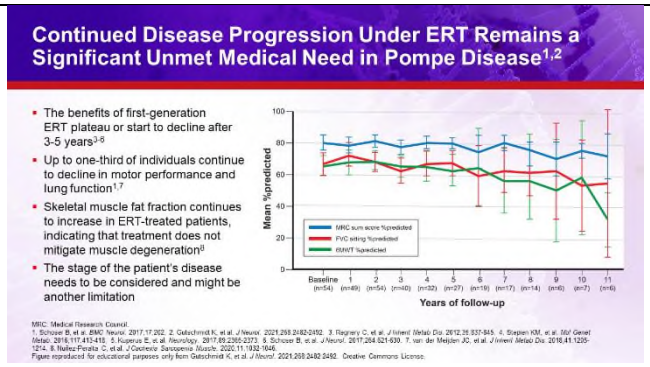
17.		So now coming back to our theme of early recognition, this is a patient who was clinically identified in childhood at age 10 months. He was started on enzyme replacement therapy, or ERT, with a glucosidase-alpha. You can see that even after being on therapy at age 3 years and 4 months, that he still has the scapular winging. He's got the lumber lordosis. He's got a head lag. And now let's see him. We increased his dose of enzyme therapy because of the persistence of these features, and you can still see that he's got continued musculoskeletal involvement. So, again, a reminder that late-onset Pompe disease can really present early. And this is not just because we're biased because of newborn screening. This is even in the setting of clinically identified cases.
18.		And so now let's look at our learnings from newborn screening. This is a baby that we saw who is 6 months old, looks pretty good, has her head up, and really is able to lie in supine as well as in prone position. But now let's compare her with a typically developing child at 6 months. This is the role and importance of the physical therapist and knowing what normal development is to compare and contrast the difference. I don't think that anyone here in this audience would say that the affected child is like a typically developing child.
19.		Now, the next is, let's look at Pompe disease as a continuum. And so on top is a baby with IOPD and one with LOPD. You can see that with enzyme therapy, there still is a Gowers' sign in these patients with IOPD telling us that this is a disease continuum. So, again, the role of close evaluation, here, you can see neck flexor weakness in a patient with IOPD. And then you can also see it in a patient with LOPD, despite being on enzyme therapy.
20.		Now let's look at some differences. And could these really reflect the extent or the impact of neurologic involvement in IOPD? I want you to focus on the child with IOPD has foot slapping gait, you know, telling us that there could be a nerve component to this disease, very different than the typical lordotic gait that we see in a patient with late-onset Pompe disease. And now let's look at another difference. Let's look at dorsiflexion and volitional dorsiflexion. I

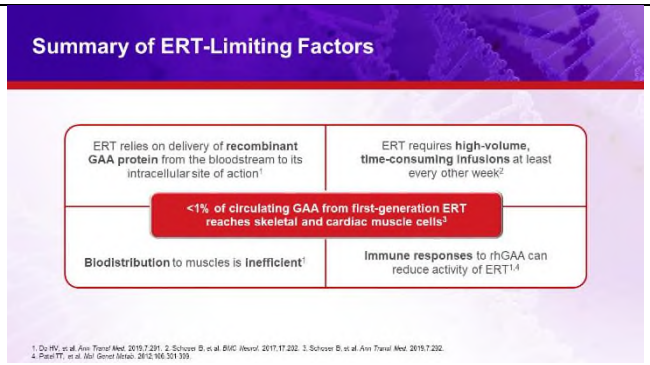
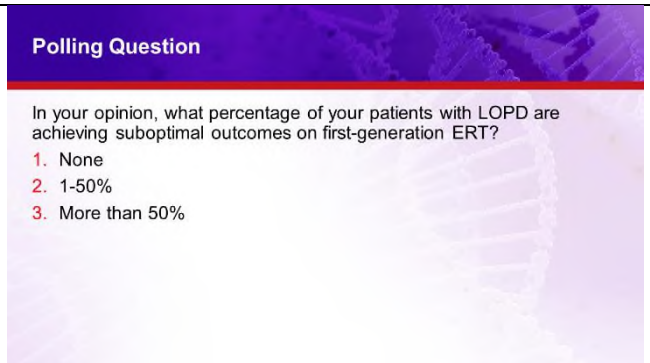
		want again to point out that in someone with IOPD, there's very early involvement of the tibialis anterior. They're unable to dorsiflex, even when you start them early—a fundamental difference as you compare it with someone with late-onset Pompe disease. So, once again, a continuum, but maybe more neurologic involvement in some cases with IOPD.
21.		Now, let's go into biomarkers. We had learned very early on that Glc_4 is a breakdown product of glycogen. It's now being used as a pharmacodynamic marker for disease severity, as well as disease monitoring and responsiveness to therapy, which you'll hear from Dr. Roberts. And so the higher the Glc_4, the more the glycogen burden. And also to remember that it could be normal in someone who's asymptomatic, but also if the glycogen is intralysosomal, because there's nothing to leak out for us to be able to see Glc_4.
22.		It's also, as I said, a pharmacodynamic marker. Here you can see the impact with increasing dose of enzyme therapy. You can see the Glc_4 coming down in this patient and now entering into the normal range. So, clearly it has a lot of utility. But you need to really consider age-related norms as you look at it and also look at trends over time, not just a single time point.
23.		Now let's look at whole body MRI. This is beautiful work, telling us that there is muscle involvement, different ways of doing the whole body MRI, but clearly from one study that in an untreated patient with Pompe, you can have an increase in the fatty infiltration by about 0.9% per year. So what does enzyme therapy do? It can slow this down by, on average, about 0.68% per year. So, once again, another utility marker that we can use.
24.		Now let's compare it to functional measures. So the Gait, Stairs, Gowers, Chair, or the GSGC, also was seen to correlate with the amount of fat fraction as measured by the proton density fat fraction or PDFF. So the higher your GSGC, that's the worse off you are, the more fat that's built up. And the same applies also with using the manual muscle testing, that the more glycogen you have as evaluated by MRI, the higher your PDFF.

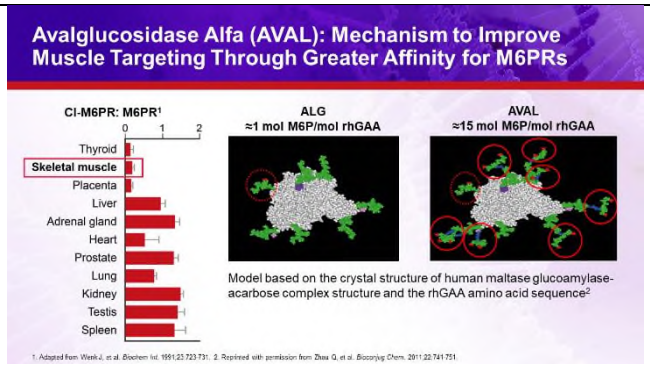
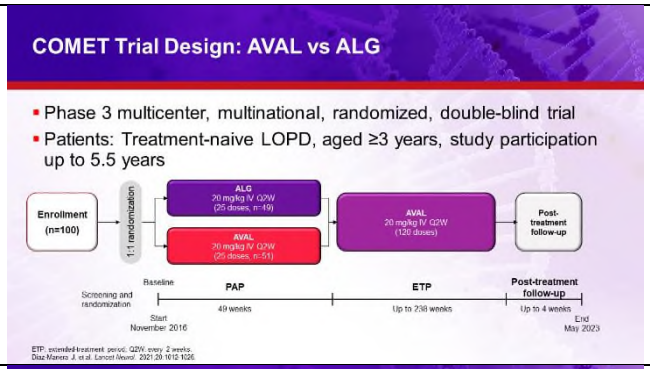
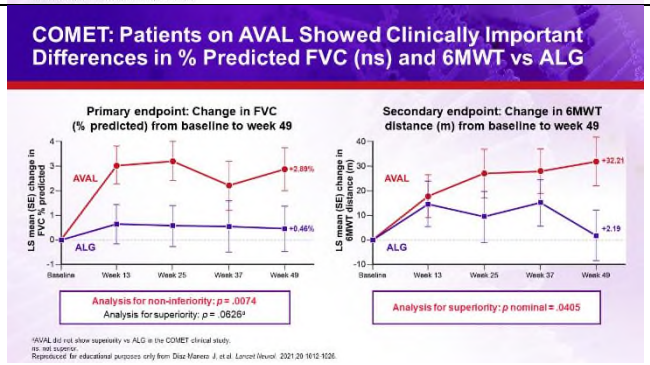
25.	**Quantitative Muscle Ultrasound and Electrical Myography in LOPD: A Pilot Study of Reliability, Longitudinal Change and Correlation With Function** ▪ Patients had received ERT for 4.2 ± 2 years on average ▪ Muscle thickness: —Deltoid, biceps brachii and forearm flexors has significant baseline values and demonstrated significant changes over the study period ▪ Subcutaneous fat: —No change at 12 or 24 months in any muscle sampled ▪ Muscle EI: —**At baseline: high echointensity (>50) for all tested muscles: deltoid, biceps brachii, triceps brachii, forearm flexors, vastus lateralis, tibialis anterior** —Stable in all muscles except vastus lateralis —The vastus lateralis EI was 77.1 at baseline, 84.5 at 12 months and 97.7 at 24 months —At 24 months was increased 27% from baseline  Hobson-Webb LD, et al. *Mol Genet Metab Rep.* 2021;28:100785. Image reproduced for educational purposes only.	And so now let's go to something that could be used more at the bedside and quicker. This is muscle ultrasound. Quantitative muscle ultrasound. Our group had published earlier about the role of quantitative muscle ultrasound in patients with LOPD, adults with LOPD. And we showed it as a response marker to enzyme therapy.
26.	**Muscle Ultrasound in Patients With LOPD Identified by NBS** ▪ 20 infants with LOPD (5-20 months old) ▪ Muscle ultrasound used to measure echo intensity ▪ Most affected muscles: **quadriceps and medial gastrocnemius** —**Elevations also seen in thoracic paraspinal muscles** —**Most frequently affected in upper extremity: biceps brachii** —Echo intensity scores correlated with increasing CK levels ▪ Utility of non-invasive imaging to assess and monitor Jackson DG, et al. *Mol Genet Metab Rep.* 2023;36:100989. Image courtesy of Priya Kishnani, MD.	So now can we apply this to the newborn screening setting? What we were very surprised to see, but not really a surprise, I should say, is that when you look at it, the affected muscles are really falling into the same group as we are seeing in adults with LOPD. And so we are seeing that this is early on, when functionally, these children are doing extremely well. And also, we found a correlation of this with increasing CK levels. And so I think it's important for us to think about using some of these non-invasive imaging markers to monitor and evaluate our patients.
27.	**The New Era of LOPD Management** ▪ New emerging phenotype of patients with LOPD ▪ Increased surveillance allows for early intervention ▪ PT is critical to identifying the earliest signs of disease progression —Kinematic analysis of posture and movement (in NBS setting) —Assessment of motor status (function, strength) ▪ Comprehensive ongoing analyses (pulmonary, musculoskeletal, biomarkers, including urine hex4, clinical, patient-reported outcomes) allows for understanding disease burden ▪ Imaging: whole body MRI and muscle ultrasound are emerging assessment tools of disease severity and progression	And so today, I really want to leave us on this note that we are in a new era of diagnosis, of management. There's a new emerging phenotype, increased surveillance. I'm not saying to over-medicalize, but also do not lose sight of some of these children. The physical therapist is your best friend. Comprehensive analysis with a multidisciplined team approach, the use of different biomarkers as available at your center, and also the role of imaging modalities. Thank you.
28.	**Evaluating the Utility of Next-generation LOPD-Modifying Therapy** **Mark Roberts, MD** *Professor of Neurology* *Manchester, UK*	And with that, I'm going to turn this over to Dr. Roberts. Thank you very much, Priya. Very comprehensive review of the pathophysiology. But our excitement about the condition is that it's treatable, a rare thing to say in neuromuscular and even in metabolic disorders.

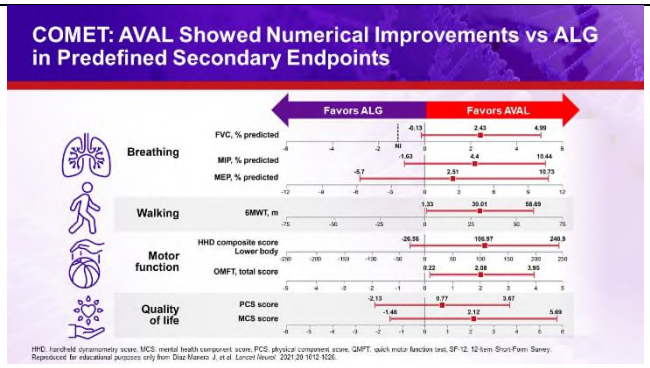
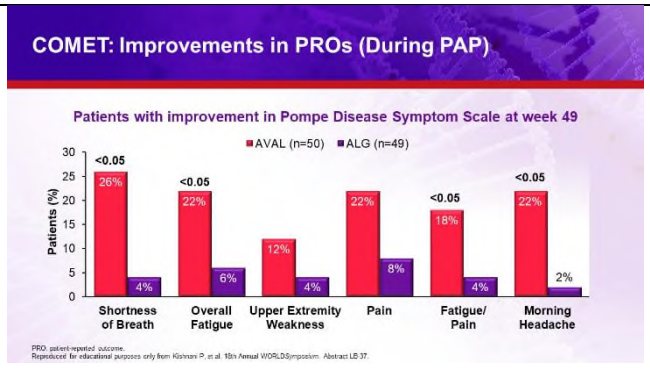
29.		So I'm just going to give you a short historical overview of how we got to where we are and how we got to the excitement of second-generation ERTs. Of course, there was crucial work in the late 60s which demonstrated the possibility of cross correction of enzyme defects, looking at cultures from patients with MPS disorders, Hunter and Hurler, and showed that at least partially using the culture medium from the relevant cultures, you could actually partially correct the enzyme defects. So this was a tremendous idea that by actually having a serological treatment, you could actually improve cellular function. And was the principle, of course, that led to the development of ERT. Of course, it was Ans van der Ploeg and Arnold Reuser who were the first to show that the intravenous recombinant treatment with *GAA* could actually partially improve the enzyme deficiency in tissues, using a very interesting bovine testicle model. They were also the first to demonstrate that this relied crucially on mannose-6-phosphate receptors expressed on the relevant target organs. And as Priya has demonstrated, of course, we're interested in the skeletal muscle, in the cardium, and potentially even in the future, the brain, though, of course, there are problems of ERT crossing the blood-brain barrier. But bovine testicles aren't going to be something that you can upscale for the human population. And it was the Chinese hamster ovary cell line developments that were crucial, and Duke was heavily involved in these, to see that this was going to be a viable treatment for patients with this progressive and indeed degenerative disorder with the first clinical trial in infants, actually back in 2001.
30.		Of course, as Ed Wraith and others in Manchester emphasized, one could never do a randomized, placebo-controlled trial in such a dreadfully progressive condition such as IOPD. But the pivotal study that Priya and others were involved with, and including patients studied in Manchester, showed that just 1 out of 62 historical controls were alive at 18 months, versus all 18 of the IOPD patients treated very early on in the course of their disease. And it was this and observations, of course, in adult patients and juveniles that led

		to the licensing of the treatment and has been available now for many years, and certainly has been the standard of care, in fact, since 2006. NOTES: • This is a Kaplan-Meier curve illustrating survival at 18 months of age in the 1602 study • On the x-axis is the age of the patient in months; on the y-axis is the proportion of patients alive • The blue line represents the 18 patients in trial 1602 and the purple line represents the untreated historical comparator cohort • All 18 patients on treatment were alive at 18 months of age compared with 1 of the 62 patients in the untreated historical comparator cohort, indicating significant prolongation of survival at 18 months of age
31.		The FDA did, of course, recommend that there should be a placebo-controlled trial in adults and children with LOPD, not least, of course, because of the profound heterogeneity of the condition. And this was the late-onset treatment study that you'll remember. This was a randomized, double-blinded study against placebo. There were 100 patients in this followed up for 1 year, and there was a useful co-primary endpoint looking both at motoric abilities—the 6-minute walk test or 6MWT, which also, of course, looks not just at motor function, but exercise tolerance—and then there's a respiratory metric, the forced vital capacity or FVC. And it was quite clear that there was a sustained improvement in these patients after an early improvement over the first 6 months, maintained out over the primary analysis period of 78 weeks. So this looks very encouraging, always with metrics. And of course, as people such as Ken Berger in the audience will agree, assessing FVC isn't always easy. So it's helpful to support this with other measurements. And certainly the maximal inspiratory pressure and expiratory pressure, useful to look at diaphragm and intercostal function as well, supported that data on the FVC, and looks so encouraging. NOTES: Pulmonary function tests have been used to clinically monitor the response of

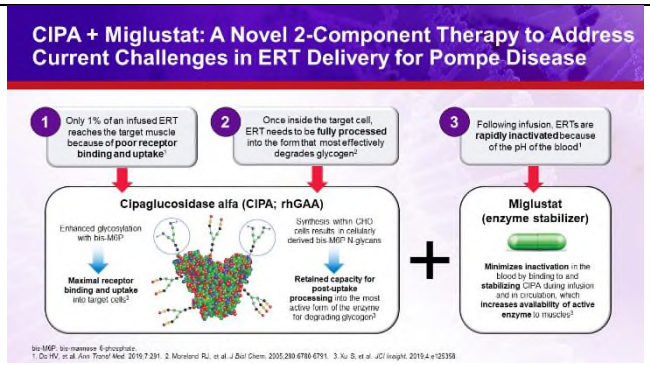
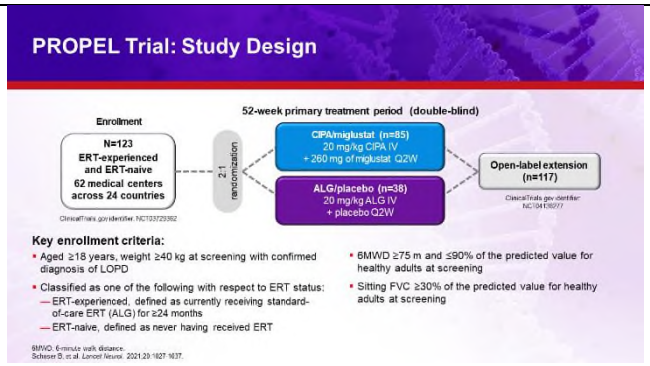
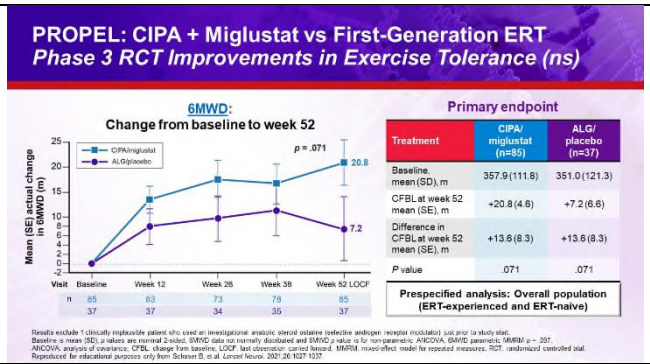
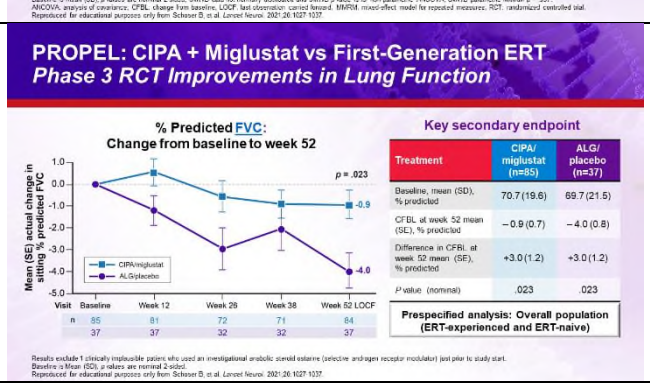
		patients to ERT. In the LOTS study, FVC, MIP, and MEP were used to monitor the response to treatment. All 3 PFTs responded. For the treated group, FVC improved 2.4%, MIP improved 9.2%, and MEP improved 9.7%. Note that these improvements were not compared with the placebo, but improvements based on the baseline values. After the initial improvements, it appeared that FVC, MIP, and MEP stabilized.
32.		But of course, the question is, with that early response in the first 6 months, will it be maintained? And of course, as you can imagine from the LOTS study, there was an extension study out to 104 weeks. And certainly in those early days, there was a profound optimism that these benefits would be maintained in patients, both with respect to their respiratory function on the left and their motoric function on the right.
33.		Now, we'd all recognize that ERT is not a definitive treatment, but it is seeking to try and ameliorate the natural history of the disease, if you like, to push the curve significantly over to the left. But early on, it soon became apparent that there were unmet needs. So, for example, some of the early infantile patients continued to have motor deterioration. And studies looking at data going over 10 years in both children and adults with LOPD, after initial improvements in stabilization in FVC and walking tests, there was unfortunately seen a progressive deterioration, and this obviously mirrors our own clinical practice and our thoughts on the patient.
34.		So we certainly see benefits for 1, 2, and even 3 years. But then, thereafter, this appears to be a deterioration in many. It's also worth pointing out that up to one-third of patients don't show an initial response at all. So again, a crucial unmet need. We've heard a lot about the complexity of the muscle histology in Pompe disease and the role of fatty change, but also, of course, fibrosis that is a progressive, degenerative and arguably irreversible scenario. So we certainly need treatments that might permeate the muscle rather better than we've seen with existing treatment, and this was shown very eloquently. Benedikt Schoser was heavily involved in this STIG study, looking at Spanish, Taiwanese, Italian and German

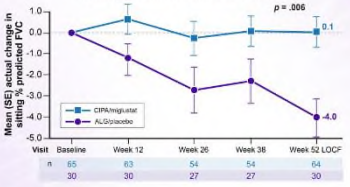
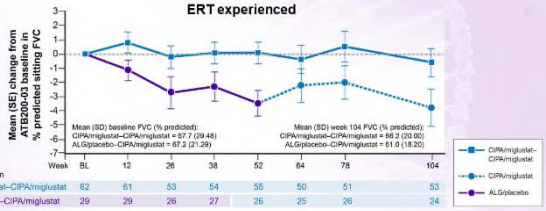
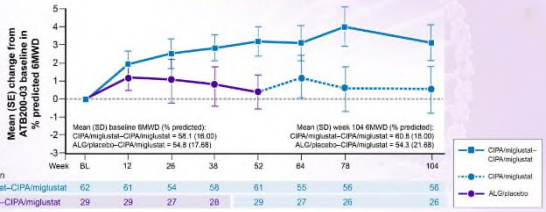
		patients for 10 or more years. And of course the numbers fall off a little toward the end. But when you look at the motor functionality, the FVC, you can see a progressive change inevitably out toward those latter years. And it's worth pointing out, of course, that so many of our patients have been on treatment with the existing enzyme for 10 or more years.
35.		So what could limit the benefit of the first generation ERT, which was obviously a game changer in metabolic medicine? Well, it requires, obviously, enzyme treatment with a recombinant protein and trying to get it in from the bloodstream into the cell, and not only into the cell, but into the lysosome. So this is challenging. One has to give a very large volume of enzyme, as you'll know, one gives in Pompe disease over 10 times the amount that one would give, for example, for Fabry disease. And it's a biweekly infusion, and therefore, quite an intrusive treatment for patients, though clearly with some benefit. The biodistribution in muscles is very inefficient, and in part, this, of course reflects the patchy involvement of muscle fibers. And we've all seen patients with both normal biopsies and later very fibrotic biopsies and complex changes in a single muscle belly. Crucially, though, of course, this recombinant protein is seen as foreign, particularly in the infants with a very low enzyme level, inherently. So the immune attack against the enzyme can certainly cause a deterioration in the benefit. So putting together all of those things, rather humbling. It's of note that less than 1% of the enzyme will actually get into the muscle cells and then into the lysosomes.
36.		So let me ask you a question: When you think about your patients with LOPD, do you feel that they're getting a good response to the current generation of ERTs, or do you think that they're starting to deteriorate? So those who feel that, in fact, their patients are very stable, perhaps a show of hands for those. Yes, not a single hand, actually. Then secondly, those who feel that their patients are deteriorating, but perhaps they've got 50% of the response they initially had, how many hands would we have for that? Yeah, we have got, you know, maybe 5 or 6 hands for that. So that certainly emphasizes that the first-generation treatment can be

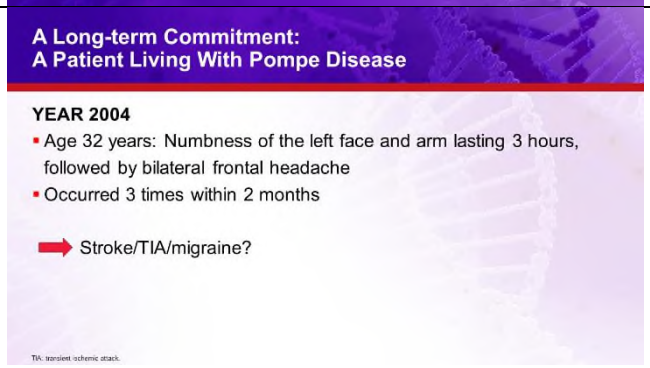
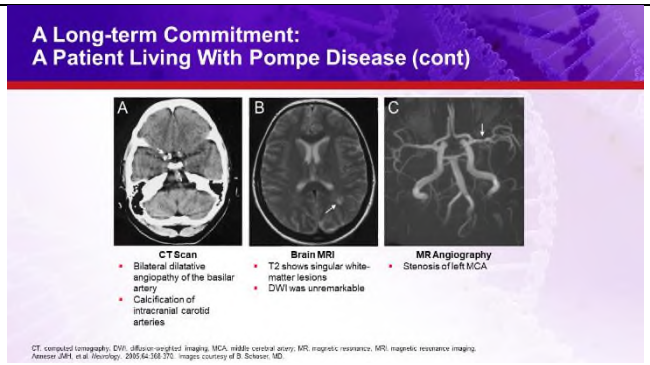
		useful still for many patients. But what about those who feel that the patients have deteriorated more than 50% relative to their initial improvement on the first-generation treatment? Yeah, and certainly a lot more hands going up for that, making a strong case for second-generation treatments.
37.		So one approach, talking about the mannose-6-phosphate receptor, is to very significantly increase the number of binding sites for that important molecule. And this is particularly important when one bears in mind that the level of mannose-6-phosphate receptors in skeletal muscle is actually intrinsically low when compared with other tissues—for example, the liver and the kidney. And this was avalglucosidase alfa or AVAL. And it shows you the significant increase in the binding sites in this novel enzyme.
38.		This was tested formally through the COMET study, a phase 3 study, which was a randomized, double-blinded study. Of note, all of the patients were treatment naive at the beginning, and they were randomized to the first enzyme produced by the same company. And then new enzyme in a 1:1 ratio, followed up then for just under 1 year, with then obviously, all patients being offered the new enzyme in an extended treatment period, going up to 283 weeks.
39.		Now, again, of course, the metrics are always complicated in clinical trials, and Pompe disease undoubtedly is a heterogeneous disorder. So when one asked whether the new enzyme was actually superior or not, it certainly was not inferior to the existing treatment, but narrowly, it missed superiority when one looked at the primary outcome, which of course was FVC in this particular study. But reassuringly, when one looked at the 6-minute walk time, there was a clear statistical benefit from the new enzyme with regard to preservation and enhancement of motor function, as judged by the 6MWT. NOTES: The change from baseline in percent predicted FVC in the upright position found a clinically meaningful improvement with AVAL, achieving the primary statistical objective of non-inferiority at a margin of 1.1 with a *p*-value of .007 and lower CI close to zero. Subsequent statistical superiority testing evidenced a *p*-value of .06. This was because the study was underpowered to detect the change, which is a

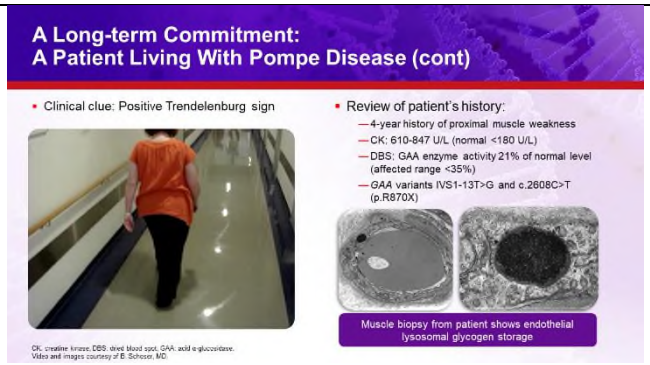
		known problem with rare diseases and small sample sizes and not driven by an absence of treatment difference. The key secondary endpoint of change from baseline in distance walked during the 6MWT also showed considerable improvement with AVAL and while the predefined testing hierarchy was broken, the difference in treatment effect between AVAL and alglucosidase alfa achieved a nominal *p*-value of .04.
40.		But of course, when we're trying to look at patients with Pompe disease, and they tell us all the time that the quality of their gait may have changed or they have less fatigue, one needs to look at other metrics which underpin, enhance, and give us confidence in the results of the primary and secondary outcome measures. So with respect to breathing, it's obviously crucial and reassuring that the MIP and MEP improved on the second-generation enzyme, that there was improvement in motor function—not just in 6MWTs, but with handheld dynamometry and timed up-and-go tests. And crucially and most importantly, that patients felt strongly using SF-36 and more specific Pompe measures that their QOL improved. NOTES: Consistency in more favorable and clinically meaningful effect was also observed across all other secondary endpoints of respiratory function, endurance, motor function and health-related QOL. Treatment effect with AVAL was numerically superior to the improvement observed with alglucosidase alfa. As shown in the back-up slides, all tertiary and exploratory outcome measures of motor strength and function, as well as QOL, also consistently favored treatment outcome with AVAL. The totality of the data clearly shows a consistent positive trend across multiple endpoints
41.		And these are perhaps the most striking data of all. So this is the validated Pompe Disease Symptom Scale. And you can see in red there the striking improvement in the patients on the second-generation enzyme versus the first, with many of these patients actually achieving very strong statistical difference, emphasizing the need to be dynamic in the metrics that we use to assess our patient response, and indeed in the future, of course, whether we switch patients.

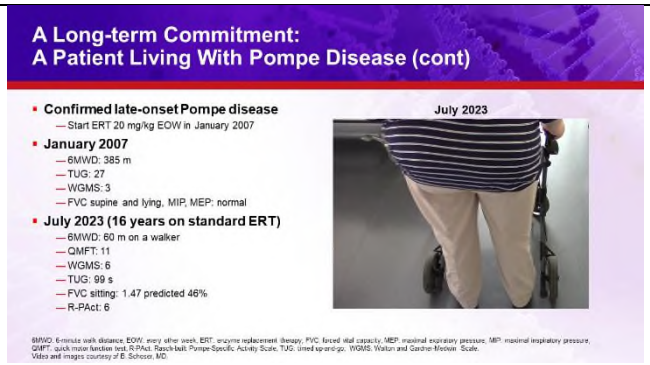
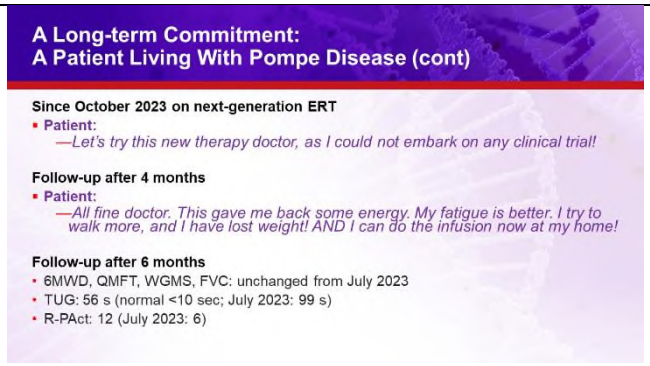
42.	**COMET: Safety of AVAL** ▪ Safety after 49 weeks —Similar IgG antidrug antibody responses in both groups • ALG: More patients with high antidrug IgG titers and neutralizing antibodies ▪ Approved as monotherapy for LOPD for patients aged >1 year —40 mg/kg Q2W in patients <30 kg —20 mg/kg Q2W in patients ≥30 kg AE, adverse event; Ig, immunoglobulin. Díaz-Manera J, et al. *Lancet Neurol*. 2021;20:1012-1026. AEs: AVAL (n=51) / ALG (n=49) Treatment: 45% / 49% Infusion: 25% / 33% Serious: 16% / 25%	So of course the question is whether this new treatment is actually safe. And there were about the same number of infusion-associated reactions, but in terms of serious reactions, a slight reduction actually in the AVAL, which was encouraging. And this led, of course, to the treatment being approved for LOPD for patients over the age of 1 year and weighing more than 40 kg.
43.	**COMET Extension: AVAL Showed Maintained Benefits and Improvements in Switched Patients (FVC)** Change in FVC % predicted from baseline to week 145 Double-blind PAP / Open-label ETP LS mean (SE) change in FVC % predicted (upright) +1.40 (1.21) +1.18 (1.32) Weeks: BL 13 25 37 49 61 73 97 121 145 Participants, n Avalglucosidase alfa arm: 51 51 51 51 49 46 46 44 43 44 Switch arm: 47 47 45 44 43 37 38 37* 36 33 AVAL arm; ALG (switch arm); AVAL (switch arm) *1 participant's FVC % predicted value at week 37 was excluded due to a physiologically implausible change between weeks 73 and 97 and 97 and 121. LS, least-squares. Reproduced for educational purposes only from Kishnani P, et al. 19th Annual WORLDSymposium. Abstract 202.	Is the benefit maintained? This was an important question, of course, from the LOTS and the other extension studies. And when you look at patients in the red who throughout had been on AVAL, and patients in the purple who were initially on the first-generation enzyme, but then offered AVAL in the extension phase, I think you'd be convinced that there's a persisting benefit from AVAL in those initially treated, but also those switched from the first enzyme have an enhanced benefit.
44.	**COMET Extension: AVAL Showed Maintained Benefits and Improvements in Switched Patients (6MWT)** Change in 6MWT predicted from baseline to week 145 Double-blind PAP / Open-label ETP LS mean (SE) change in 6MWT distance (m) +20.65 (9.60) +0.29 (10.42) Weeks: BL 13 25 37 49 61 73 97 121 145 Participants, n Avalglucosidase alfa arm: 51 51 49 50 48 47 47 44 44 45 Switch arm: 47 47 45 45 43 43 41 41 32 35 AVAL arm; ALG (switch arm); AVAL (switch arm) Reproduced for educational purposes only from Kishnani P, et al. 19th Annual WORLDSymposium. Abstract 202.	This is also seen when one looks at the 6MWT, a clearly maintained response to AVAL, but reassuringly in those patients initially treated with the first-generation enzyme, shown in blue, who appeared actually at 1 year almost to be about to deteriorate, then a stabilization occurred when switched to the new product.
45.	**Limitations of Current ERT** IMPAIRED DELIVERY Limitations of inherent anatomy Inefficient transcytosis leads to poor ERT distribution in the interstitia Nearly all patients with Pompe disease develop anti-rhGAA antibodies rhGAA unstable at neutral pH, leading to degradation and loss of ERT in circulation "Sink effect". Substantial rhGAA clearance from circulation by nontarget muscle tissues (eg, liver, spleen, lymphatic system, GI tract) Vascular endothelium Muscle fibers SUBOPTIMAL UPTAKE Cellular uptake of ERT is via receptor-mediated endocytosis M6P required for efficient cellular uptake rhGAA is poorly phosphorylated, limiting ERT uptake by target muscle cells Blood space Interstitial space Anti-rhGAA antibody rhGAA ERT Receptor Figure reproduced for educational purposes only from Do HV, et al. *Ann Transl Med*. 2019;7:291.	Just to come back again to this problem about the access of the ERT to the relevant tissues. Multiple factors of course are relevant. Fibrosis, patchy muscle involvement, the fact that you're actually giving a treatment intravenously, a long way actually from the lysosomes deep in the skeletal muscle cells, patchy distribution of mannose-6-phosphate receptors can be relevant. And of course one can view that the blood is actually a relatively hostile environment in a pH basis for the enzyme.

46.		So another approach with another second-generation ERT is actually using a novel 2-component treatment, again with the enzyme having enhanced mannose-6-phosphate receptor systems, and to reduce aberrant abnormal phosphorylation, but also using an enzyme stabilizer given orally in the hope of stabilizing the enzyme in the circulation to enhance the amount that therefore might reach the target tissue of muscle, and perhaps also with some additional effects within the cell, getting through to the lysosome as a chaperone.
47.		This, of course, was then studied, at its time, the largest ever study actually in Pompe disease, the PROPEL study. This was 123 patients. These were patients who were naive to all treatments, just a few patients, actually. The majority of patients had been on the standard of care for many years—in fact, the majority over 7 years on treatment. And they were randomized to either remain on the current treatment or to go onto the new treatment, CIPA (or cipaglucosidase alfa)/miglustat, as the combination therapy with then an open-label extension after the primary analysis period.
48.		Looking at the data, and this is looking at the overall treatment population, both those switched and those who were initially naive to treatment. And you look at the primary outcome measure, the 6MWT was chosen. You can see that although there certainly was a trend toward an improvement in those treated with CIPA/miglustat, unfortunately, didn't quite reach statistical significance.
49.		By contrast, when one looked at the secondary endpoint, namely the effects on respiratory function, you can see in the blue line here a preservation of FVC in those on the second-generation enzyme versus the expected deterioration seen in those who are on the first-generation enzyme. And this did reach statistical significance.

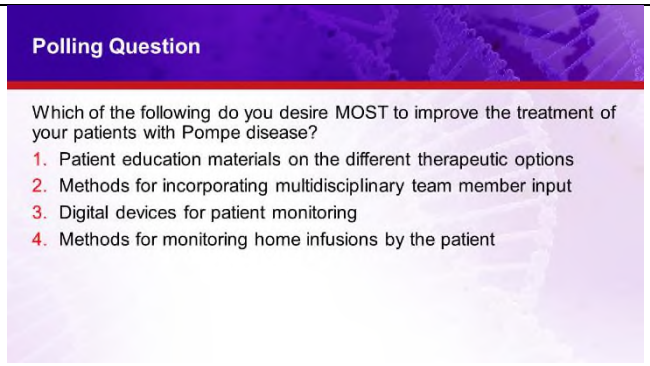
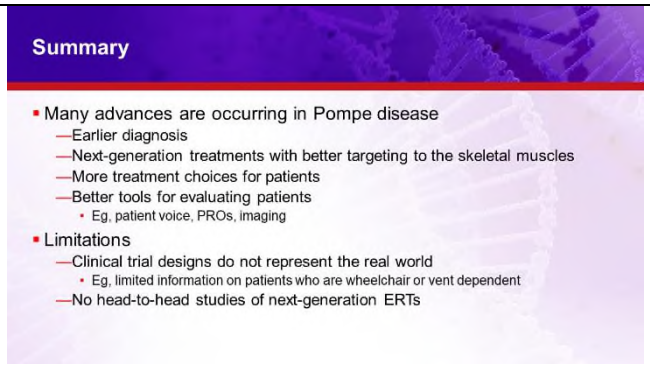
50.	**PROPEL: CIPA + Miglustat vs First-Generation ERT *Phase 3 RCT Improvements in Lung Function—SWITCH Patients*** % Predicted FVC: Change from baseline to week 52  Key secondary endpoint Treatment: CIPA/miglustat (n=85) vs ALG/placebo (n=37) Baseline, mean (SD), % predicted: 67.9 (19.1) vs 67.5 (21.0) CFBL at week 52 mean (SE), % predicted: + 0.1 (0.7) vs – 4.0 (0.9) Difference in CFBL at week 52 mean (SE), % predicted: +4.1 (1.2) vs +4.1 (1.2) *P* value (nominal): .006 vs .006 Prespecified analysis: ERT-experienced	Focusing on the majority of patients who were actually switched from one enzyme to the others. They were experienced patients to ERT, you can see that the FVC difference between those who are switched and those who are actually kept on the older treatment is even more striking and has a higher level of significance.
51.	**PROPEL: Safety of CIPA + Miglustat** Safety after 52 weeks AEs: CIPA + Miglustat (n=85) vs ALG (n=38) Treatment: 95% vs 97% Infusion: 25% vs 26% Serious: 9% vs 3% ▪ 2 patients withdrew from CIPA + miglustat arm due to infusion-associated reactions, one of which was a serious AE ▪ Overall, safety profile of CIPA + miglustat is similar to ALG	And what about safety? Well, infusion-associated reactions at about 25% certainly were about the norm and very similar to the first enzyme. There were actually a few serious side effects. And this makes the case, of course, that although many countries have a big and very well established home care therapy program, certainly in the early months, those patients need to be assessed in hospital, just in case of these serious reactions.
52.	**PROPEL EXTENSION** **ERT-Experienced Population: FVC Outcomes** Sitting % predicted FVC ERT experienced  Participants, n CIPA/miglustat–CIPA/miglustat: 62 61 53 54 55 50 51 53 ALG/placebo–CIPA/miglustat: 29 29 26 27 26 25 26 24	Focusing on whether this response is maintained, and these are in ERT-experienced patients rather than the overall population, you can see that in those treated initially with AVAL shown in the blue, as well as those who were actually on the standard of care shown in the purple, that there was a maintained response with regard to the vital capacity. That is very encouraging.
53.	**PROPEL EXTENSION** **ERT-Experienced Population: 6MWD Outcomes** % Predicted 6MWD  Participants, n CIPA/miglustat–CIPA/miglustat: 62 61 54 56 61 55 56 56 ALG/placebo–CIPA/miglustat: 29 29 27 28 29 27 26 26	In patients who had been throughout on the new product, you can see a maintained response in the 6MWT. Crucially, we were starting to see an early deterioration potentially in those on the standard of care shown in purple, but there appeared to be some recovery, or at least stabilization when they were switched to the new enzyme CIPA.
54.	**Conclusions and Discussions** ▪ Fantastic to have 3 ERT choices! ▪ Second-generation ERTs will be important long-term bridge to definitive (gene therapy) treatments ▪ Other combinations, including SRT, and gene therapy may be useful ▪ Treatment decisions will be informed by clinical trial data, real-world data, and patient preference ▪ Clinical trial outcomes suggests multiple metrics, including PROs, will be important	So I think putting all the totality of these early clinical trials data together, including the extension studies, I think it's clear, it's fantastic to actually have 3 treatment options for patients. But I think we'd all recognize that second-generation ERTs will be a very important, and I suspect long-term bridge before we might get to gene therapy in the future. It's likely given that the benefits in some patients are relatively modest, that it would be really helpful to think about combining

		treatments with ERT, with substrate reduction therapy, and even gene therapy in the future as that becomes more readily available. And we have to think carefully about our treatment decisions, both on these trial data, but also real-world data as they increasingly become available. And patient preference as well is going to be very important. And not least, we have to think about multiple metrics, including importantly, patient-reported outcomes, as we make these treatment decisions and these treatment change decisions. Thank you very much, and I'll pass to Professor Schoser.
55.		All right, so good morning. So what can I add after these super presentations? So, first of all, I'd like to thank you for your commitment to come out here this early and listen to us. And I think that's really a major issue here. I also like to show you a commitment, a long-term commitment of one of my patients.
56.		So it's a patient living with Pompe disease now for at least 30 years, and I got to know her 22 years ago. So it's really a long-term relationship between a patient and a treating physician. It all started in 2004, so it's really a long time ago, 20 years, and at that time she was 32 years old, presented at the emergency department with numbness of the left side of her face and her arm lasting for 3 hours. This was followed by a frontal headache. Episodes happened 3 times within 2 months. So that's why she was admitted. And of course, there was a differential. Is this a stroke-like situation? Is this a migraine or even a seizure situation?
57.		And therefore, the people at the emergency department, they did first line in the night a CT scan, and here they already realized there was a dilatation of the basilar artery and also noticed some calcification. And in the morning, they decided to go for an MRI of the brain and an angiography. And there they definitely saw some white-matter changes were there, but very tiny little lesions. But, of course, it was a so-called dilatation of the basilar arteria, what we call the megadolicho basilaris phenotype. And there was very low blood flow in this. And that was really the situation for this patient.

58.		But then, in the routine laboratory testing, they realized there were some more things going on with the patient. There's a mild proximal paresis that was not related to the situation that brought her to the emergency department, and there was also a CK elevation. And therefore, they referred the patient to me for a workup for the hyperCKemia. And what I realized, and hopefully the movie is starting, which was taken a bit later—there was already a classic waddling gait. So there was a Trendelenburg in that patient, proximal weakness, and also the arms were not as strong as they should be. And we had the opportunity, at a very early stage, to evaluate for *GAA* activity, but it took 8 weeks to get results. And so with a workup for hyperCKemia, we did something very classic. We used a muscle biopsy, and it looked for enzyme activity in the muscle tissue and also for the glycogen content. And, of course, it turned out it was a vacuolar myopathy. And all these types of things—the enzyme was reduced, the glycogen was high, and later on, with the sequencing of the *GAA*, we found a very classic Sanger sequencing. So this took more time than it does nowadays. It was a similar situation. And what we realized here was that even in the vasculature of these patients, in the muscle tissue, there was already a glycogen deposition. And that was later on, very well worked out. And we did know this information already. So it was not brand new. It was already there, if you look carefully at autoptic cases from the 1960s and 1970s and the angle. So one of the icons of Rochester University worked on this and already described parts of this. So it was all there. But it was, of course, neglected in a way. We were all focused on the metabolic part of the disease, but not on the multisystemic thing. And so therefore, we thought, it's good. And we published this in 2005. So it's really a long time ago.

59.		So what happened? So as of 2023, this patient has received 400 standard-of-care infusions. So consider this—this is about 7 million US dollars for a patient over time, just on the enzyme. And here you see the decline. So in 2007 she was able to walk 385 meters in 6 minutes and the times up-and-go was 27. And also that WGMS grade was 3, but still the lung function was preserved, so that was all fine. And later on, you see, she was mainly sitting in a wheelchair and on a walker could just walk 60 meters and had a steady decline in all other motor functions. And on top there was also a big steep decline in the pulmonary function.
60.		Even in the walking you saw that there is a much prolonged and very severe disease now. This type of patient will never ever go to a clinical trial, she was never able based on the exclusion criteria to go on one of these phase 2 or even phase 3 trials. So therefore we had a long-term relationship and discussed several times what to do and what not to do. And in October she came up and said “Well, come on doctor, let's really try one of the new treatments.” So I could never embark on any of the clinical trials, and now we have licensed drugs, so why should I not go and have a try? And finally I said “Yeah, why not?” So it’s time to move on. And if there's still a functionally preserved tissue at risk, you can treat. So why shouldn't you switch a patient to one of the new enzymes? And that's very important. And that's what we did. And of course this is a very short-term follow-up. There's of course now no new change, but there were also no new side effects, but she was already commenting—and that's the point I'd like to make here—that “Well, this gave me back some new energy,” “My fatigue is better, so I feel better, I even think I can walk a few steps more,” and something else that happened to all of these patients, “I lost a little weight again.” Because if your food intake stays the same, but your muscle capability to walk, to perform exercise declines, you gain weight easily, then you pick up a lot of extra pounds. And that is one of the issues here. And with this, at least it was for her the first time that she said, “Well something is changing.” And what was the message here of this “you gave back hope”. And hope is really transferring into also a functional commitment that she's now doing again all the

		testing that we are doing with her and also has the commitment to go very regularly now on the new treatment. And we will see what really happens to this patient in the long-term. But this is something very important. So adding a second type of ERT, a second-generation ERT is a very important decision that you have shared with the patient. So framing really, what are the expectations? What can we hope for together if I put you on a new treatment? And I had here the lucky situation that I put also a second-generation physician, treater physician on here. So I have here in the room Stefan Wenninger, a dear colleague of mine, and he is now committed and continues to work with the patient. And also this adds something. So it's the personal relationship in very chronic diseases that is so important. And you need to talk again and again to your patient. What is the exact right time point to switch? And of course you have all the knowledge, but the patient community, don't neglect them here in Pompe disease. They are very well aware of our clinical data and they know to read this right now. So therefore we need to keep them informed and try to get this message out together. And then it's not only hope, it's not only an emotional rescue, it also transfers really into a new treatment perspective of a very individualized basis, and that's very important.
61.		So therefore, in a way, what we are doing, we face a shift in Pompe disease in decision-making. We have a lot of committed patients. We have a lot of committed first-, second-, and hopefully third-generation physicians who work with us. And also, of course, the industry is needed for this. We have now this early diagnosis, especially over here in the US, with the newborn screening. That also changes part of our thinking. We have the growing role for this decision-making, and we need to create together some so-called lay language material that really is a fair-balance option for discussing all the different treatments we have. We also need to integrate a multidisciplinary team that really has a very holistic view on the patient. And then that's one of my hopes. And you listened to this yesterday morning, that we need this digitalizing next step in our patient-reported outcomes, and in all our clinical trial designs—we are still stuck in the last century with how we

		are reporting. So 6MWT and all this, this is really testing from the past. We need to move on for our clinical trials, for new things, and that's here. And another thing that was perhaps not a big point in the US, but for all these treatments now in Europe, you have the option of home infusion. We know about the risk of this a bit, but anyhow, it's really a new way for the patients to have this treatment in their home. Also, they need to be monitored very safely. That's another thing we do not neglect over time. So we need to follow-up with them and know a communication pathway has a strategy to build up all these things we need to really use this type of approach.
62.		So now I also have a polling question for you. Which of the following do you desire most to improve the treatment of your patients with Pompe disease? There are 4 answers here. Patient education materials on the different therapeutic options, methods in incorporating multidisciplinary team members, input digital devices for patient monitoring, or methods for monitoring home infusion by the patient himself. So again, who is voting for number 1? Good. Who is for number 2? Yeah, okay. Number 3? Yes, good. And methods for monitoring, home monitoring.
63.		Okay, so let me wrap up our symposium. So we had 2 super speakers. They really brought up the new ideas we have for this disease. I think that's very good. We are diagnosing earlier. We have now 2 types of next-generation treatments. They are better at targeting skeletal muscle. We have still the caveat of the CNS. I think we need more treatment choices for the patients, and we have at least now 3 choices. So that's very good. But I guess we will have in the future only 2 of these ERTs, finally. Anyhow, we need to have better tools for the evaluation of our patients. So we need to integrate a bit more. Again, patient voices, PROs, and have more work on imaging. I think that is neglected. We still need to do more there, and we still have some minor limitations. I feel the clinical trial design is not really perfectly transferring into the real world. So that's a line up in a way, if we look at the preclinical data sets to the clinical trials and then clinical trials to the real-world situation. This is, unfortunately, always a decline in the efficacy of what we are doing and we need to rethink. So

		what is really the part where we have to do more work and where do we need extra thoughts? Young, fresh brains also needed there. And of course, we don't have this type of head-to-head study with our next-generation ERTs. But I think some part of this can be done in the registry studies that could really do some similar work on the 2 new enzymes. And then we will have more insights and perhaps even next year we can present the first results on this.