Ozlem Goker-Alpan, MD

Welcome everyone to the CME series on lysosomal disorders. This is the second presentation for this year, and we are delighted to have Dr. Neal Weinreb. Before I introduce Neal, I will just briefly summarize the cell biology of the lysosomal proteins, and how we ended up actually designing the treatments. So in 1974, Dr. De Duve, a Belgian scientist and his associates received a noble prize for description of the lysosome. To exact that their observation was the cells released and enzyme called as acid phosphatase in a much larger amounts, when they were repeatedly frozen and thawed before centrification. So they described this very important discovery and in the same publication, they proposed a therapy option, which is the replacement of the missing enzymes, if that is the case. So when we look at the lysosomal protein or lysosomal proteins in general, they are mostly enzymes.

They are found in the lysosomal membrane. And they have quite long half lives, about 72 hours or more. Which makes them vulnerable to the events, including, misfolding and degradation. So lysosomal enzyme C gets synthesized in the ER, and needs to reach the lysosome. Any mutation that renders the lysosomal enzyme, either actual degradation or not reaching to the lysosome actually will cause either a real or functional deficiency of this protein. So multiple other molecules in the cell, such as the ER chaperone families, actually have significant contribution to the folding of a lysosomal protein. And after the proteins are synthesized, they need to be targeted to the lysosome, which is one of the most crucial steps. And as Dr Weinreb will talk about enzyme replacement therapies, this was the seminal step to understand the enzyme replacement therapy, as the initial attempts about understanding of the role of mannose receptor, and mannose 6 receptor mediated pathway. The initial enzyme preparations, actually that ended up in the liver for enzymes until the manuals residues were in introduced as the recognition events.

So, obviously that we discussed the disease causing mutation, which can be actual subject to the cellular events. And then either there is a functional or an actual real deficiency. So basically when there is loss of function, we can talk about replacement therapy. And when there is the substrate formation and deposition, you can address this with other small molecules, such as SRT, and which is also enzyme enhancement therapies, AKA chaperon therapies. Still there is complex cellular pathology, and there are diseases have a best pairing pheno heterogeneity, even within the same genotype. There are problems delivery to all systems. CNS is a major organ that is actual resistant to the ERT, or even the other small molecule therapies. And obviously when we are looking at the complication phase of all these disorders, there is the inflammatory component. So on today's lecture, we're going to be covering on ERT and small molecule therapies, especially substrate reduction therapy.

And with this brief introduction, I would like to introduce Dr. Neil Weinreb. He has been in the field since 1968. Actually he's the first author on the paper that describes the location of the sphingolipids, the enzymes that break down Sphingolipids on the lysosome membrane. He has been a fellow with Dr. Roscoe Brady, where after which that he moved to Mount Sinai. And if after a few years, he had been at the university of Miami. Currently, he is the director of the university research foundation for lysosome almost storage disorders since 1996. And he has been actually one of the founders and also of the board. And which is current is on the scientific board of the international collaborative Gaucher group and Gaucher registry. He has published almost a hundred papers on lysosome disorders with a special interest in Gaucher disease. Neil, it's my delight to have you as a speaker today, please start your lecture. Thank you.

Neal Weinreb, MD

Thank you very much for that kind introduction. Today, I'm going to discuss lysosomal storage diseases, in terms of an update on both enzyme replacement therapy and substrate restriction therapy, for a number of these interesting disorders. The learning objectives are to describe how ERT and SRT have transformed the lysosomal storage disease population and treatment of these disorders. Who also describes some new research underway to improve the safety and efficacy of the existence, ERTs and SRTs. And finally, to touch on how ERTs and SRTs are addressing the problem of the blood brain barrier. Obviously this has become a very large subject, and I'm only going to be able to touch on certain highlights and certainly will not be able to cover the breadth of what's been going on with treatment of LSDs. And the next slide I'd like to go over a bit of history of the development of treatment for the lysosomal storage diseases.

And I will use Gaucher disease as a model because it was actually the first LSD for which treatment was approved, about 30 years ago. So you can see that the disease was first described by Philip Gaucher in 1882, and later on in the 1960s, my mentor, Dr. Roscoe Brady investigated the biochemical cause of Gaucher disease, and found that the storage of the glucocerebriside, which had been described back in 1932, was attributable to a defect in the breakdown of that particular chemical. By the enzyme glucocerebrosidase, which was shown to be deficient in patients with Gaucher disease that he studied. From that point on, development was considerably more rapid, Dr. Brady succeeds in purifying a small amount of the enzyme in the early 1970s, and actually tried to administer it to a small number of patients, but that attempt did not work out. And subsequently, it took further information, including the ability to synthesize larger amounts of the enzyme, and also to modify the enzyme by correcting its carbohydrate structure, to enable it to be taken up by a cells.

And that actually took place during the late 1980s. Was at that time that an ID was then put forth for the enzyme that was synthesized by Dr. Brady, which eventually was approved on the basis of a 12 patient trial in 1991. And that actually started the era of enzyme replacement therapy. The next slide is a cartoon representation of the basis for the two major treatments that I'm going to address today, namely the various enzyme replacement therapies for lysosomal storage disorders. Which are all primarily based on the idea of replacing or augmenting the enzyme therapies associated with the various lysosomal storage disorders. However, as you can see, again from this cartoon, there is a second approach, which is based on the idea of reducing the amount of the effective substrate that is synthesized.

And in so doing enabling even a relatively defective degradative enzyme to work more efficiently, in terms of preventing storage and buildup of the substrates, which ultimately lead to these lysosomal storage disorders. Again from a historical viewpoint of the next slide, you'll see that in 2013, there were a number of enzyme replacement therapies, which have been approved for various lysosomal storage diseases, including Gaucher disease type one, and the neuropathic form type three. Fabry disease, which had two products approved and only one in the United States. Pompe disease, the glycogen storage disease, which alglucosidase alfa had been approved, and a number of the mucopolysaccharidase storage diseases, namely MPS one with its varying forms, Hurler disease, Hurler Scheie disease and Scheie disease, had approval of an enzyme,

Iduronidase. MPS II or hunter syndrome. Also, iduronidase-2-sulfatase had been approved for MPS six. Another enzyme therapy galsulfase had been approved. And there was also another product cysteamine bitartrate, which was a treatment for stenosis, a type of substrate reduction therapy. Additionally, Gaucher disease by this time, and one of the initial substrate restriction therapies had been approved. So this is where things were at in 2013. Within the last couple of years, a number of new products have been approved, a new substrate restriction therapy, Gaucher disease known as eliglustat, which I'll discuss in a little bit more detail. A different type of therapy called chaperone therapy for Fabry disease

called migalastat, which I'll again, speak about a little bit later. A updated enzyme replacement therapy for pompe disease called avalglucosidase, which was just approved about approximately one year ago. And then a couple of new treatments for some of the other mucopolysaccharide storage diseases, namely MPS IV A, and MPS VII

Now, again, since we are also going to deal with some of the other advances, it's important to also point out that there are quite a number of different clinical trials, which are in progress. Which involve a number of different mechanisms for therapy, an enzyme therapy for acid sphingomyelinase deficiency. Or Niemann pick a and B, for Niemann pick B was just approved an enzyme, which is known as olipudase. And this was actually just something that should place a couple of weeks ago on the basis of trials, even though this enzyme has been studied for a long time. And you'll notice that many of the current treatments of also switched to the arena possible gene replacement therapy, or gene correction therapy. Which is not a subject that I'm going to deal with today. During the last three years, it's also, I think of note that there have been quite a number of orphan drug designation IDs entered with regards to the lysosomal storage diseases.

But again, I think you'll see that the majority of these deal with the newer approach of gene correction therapy, which actually has not yet really entered into the commercial clinical arena. It's also interesting, I think to note that since the start of the orphan drugs program in the United States, back in the 1980s, there have been a total of 5,366 INDS, submitted for these rare diseases. But only 150 INDs have been for lysosomal storage diseases. And you'll see that so far only a few of these have actually relative to the number of applications resulted in approved products that are being used.

So with that background, let me turn to some illustrative lysosomal storage diseases. First Gaucher disease. Gaucher disease is one of the most common lysosomal storage diseases. It is transmitted and auto solo recessive fashion. And it is almost always associated with mutations of the GBA-1 gene. The disease is associated with storage of glucocerebrosidase, predominantly in the monocyte macrophage system.

And for this reason, it is usually associated with systemic manifestations rather than with CNS manifestations. And it comes in three major classifications. The most common one in Western countries being type one disease, which has generally been classified as being non neuropathic, except for the fact that we now know that later in life, can be associated with Parkinson's type disease. And then there are the two types of neuropathic disease. The so-called acute neuropathic type II, which is a disease which occurs anywhere from birth to early in childhood, and which is invariably fatal early in life. And then the type three or product neuropathic disease, which can affect children and infants as well, but which I can sometimes be associated with a much more prolonged survival.

The major disease manifestations are related to storage of the lipid glucocerebrosidase and its secondary substrate, lysosome GL-1 in primarily disease cells of the particular endothelial system. And this storage is associated with chronic inflammation and antigenic responses to an antibody responses to the glycolipids, which can contribute to an increased risk for certain malignancies and prominently multiple myeloma. Because the bone marrow contains a number and a fairly large population of macrophages and particular endothelial cells, the skeleton is also a significant target of pathology in individuals with Gaucher disease.

We now have two treatments primarily for Gaucher disease, which are commercially available, two treatments conceptually, more treatments as I mentioned before, in terms of specific products. Enzyme replacement therapy exists in three forms. Three enzymes, which I alluded to before. And this involves infusion of recombinant modified forms of glucocerebrosidase, which are targeted for uptake by macrophage mannose receptors. The three products in imiglucerase, velaglucerase and taliglucerase are usually administered by IV infusion, typically every two weeks sometimes in different infusion schedules.

And can be administered not only in the clinic, but also via home infusions. The results of all three products are basically similar. There's been improvement in hemoglobin and platelet counts, which are usually abnormal in individuals with untreated disease reduction in large liver and spleen volumes, which is also a characteristic abnormality in patients untreated with disease. Improvement in bone mineral density and improvement in osteopenia and osteoporosis. Decrease in symptoms of bone pain, and improvement in general, quality of life, enhanced activity levels and improvement in lifestyle. Before treatment with enzyme face and therapy was available,

Patients usually required treatment with splenectomy, which unfortunately actually made the phenotype of disease portion, and only succeeded in improving some of the hematologic manifestations of the disease. Unfortunately, enzyme replacement therapy is not perfect. Although it is extremely effective and has been associated with a marked change in quality of life for patients who receive it. But there are some limitations to enzyme replacement therapy for Gaucher disease, because the treatment is invasive, it can sometimes become inconvenient, or difficult to administer if venous access is poor. Unfortunately because the enzyme is a large molecule that can't traverse the blood brain barrier successfully, the treatment has not been effective for the neuropathic forms of Gaucher disease. It also has not been clearly shown to prevent the emergence of the Parkinsonism that can occur in later life in individuals with type one Gaucher disease. And it also appears to only in completely control some of the macro age activation effects, which can lead to cytokine activity, T and B cell stimulation and various inflammatory manifestations.

And finally, the skeletal manifestations because the uptake in bone can sometimes be limited, and has not been as successful as the treatment of the enlarged liver and spleen and hematologic manifestations. And we know that even patients who appear to be treatment successfully in terms of their systemic manifestations, can occasionally still go on to develop bone complications despite being on effective doses of the treatment. This next slide, you'll note that in individuals who are normal, almost all of enzyme, which was administered during clinical trials, was taken up in the liver and in spleen, in individuals with the Gaucher disease. Again, most of the uptake of the administered enzyme does take place within the liver and spleen, and only relatively smaller amounts within the bone and within the bone marrow, which probably explains why enzyme treatment has not been 100% successful. As a result of the limitations of this therapy,

There was interest in developing alternative types of treatment for Gaucher disease. As I indicated before, substrate restriction therapy through inhibition of the glucoceramide synthase enzyme, which is responsible for production of the ultimate storage product, has been investigated. The earliest product, eliglustat, that has actually been replaced by a newly approved drug, which was approved by the FDA in the United States in 2014. A product known as eliglustat. Eliglustat is a small molecule. So consequently it is not limited in its activity to uptake within monocytes and macrophages. And it may therefore be active in other cell types, which can be affected in Gaucher disease. Studies have suggested, and I'll go over a few of the studies in a little bit more detail, that it's efficacy seems to be roughly comparable and certainly not inferior to enzyme replacement therapy. It has a pretty good safety profile.

Eliglustat, which is somewhat unique, is that as a small molecule, it is metabolized primarily by liver enzymes, and most specifically CYP2D6. And consequently it's dosing is dependent on the degree of activity of those enzymes and the genotype for CYP2D6, which determines whether somebody is a normal metabolizer of the drug, or may be a poor metabolizer. In which case for a given dose, there is a possibility that one could have a higher blood level leading to, potential toxicity and conversely patients who are ultra rapid metabolizers don't achieve therapeutic blood levels. So in order to administer this drug successfully, one has to test the target patient for the CYP2D6 genotype.

And that will determine whether the drug is appropriate and in what dosage it can be taken. Now, unfortunately, even though this is a small molecule eliglustat, does not remain within the CNS. It's extruded from the CNS by PGP drug transporter. And therefore it is also, unfortunately not an answer for treating individuals with neuropathic Gaucher disease. Eliglustat actually was investigated in a very large series of clinical trials. Which involved treating both go treatment naive patients, as well as switching patients from enzyme replacement therapy to the eliglustat. And some of the general findings revealed that in treatment naive patients, there was a rapid and significant decrease in the size of liver and spleen, improvement in hematologic parameters specifically in terms of thrombocytopenia and anemia. Achievement of various other therapeutic goals, and improvement in bone parameters.

And in patients who were switched from enzyme replacement therapy to eliglustat and who were studied in various larger trials . In the two trials that were conducted in treatment naive patients, there was noted to be a somewhat more rapid and perhaps even a more extensive improvement, in bone mineral density that had previously been observed in patients treated with enzyme replacement therapy. And in terms of certain biochemical markers, which have been correlated with the severity and persistence of bone disease in this particular slide, illustrating macrophage, inflammatory protein, one beta. You'll notice that for a treatment naive patients, there was rather impressive normalization of the biomarker and in patients who were switched from enzyme replacement therapy and who have had improvement in the biomarkers on that, it was maintenance of biomarker stability within the normal range. In the real world, eliglustat is also proven to be as successful as in the clinical trials.

And in this study, which came from a single center at Yale, it was observed that after two years of eliglustat, the treatment naive patients had clinically meaningful and statistically significant improvements. And the switch patients remain stable with respect to hemoglobin platelets, spleen and liver size. And in fact, although the data was somewhat limited, there were actually some evidence suggesting that biomarkers, which had improved on enzyme replacement therapy, may have improved even further after patients were switched to Eliglustat therapy. Now, as I mentioned, we still don't have any satisfactory treatment as of yet for the neuropathic forms of Gaucher disease, but a newer next generation substrate reduction or substrate restriction therapy known as venglustat that is in clinical trial for patients with type three Gaucher disease.

This particular substrate reduction therapy does successfully cross the blood brain barrier, and effective concentrations have been measured in CNS tissues, in preclinical trials, in neuropathic Gaucher mice models. Venglustat was shown to lower the synthesis of the glycosphinglipid to reduce accumulation of the glycolipids and liver and spleen, to cut down on some of the brain inflammation, evidence by gliosis. And also to improve some of the clinical manifestations observed in these mice models, specifically ataxia and paresis, and increase the lifespan by about 30%.

There's currently an ongoing trial known as LEAP, which is studying and in phase two trial, the use of Venglustat along with continued enzyme replacement therapy with imiglucerase in initially adults, but now subsequently in children with the disease who are now being enrolled in the trial. I don't have any recent results to show, but at meeting in 2020, some early results in some of the adult patients were presented, which indicated that there was a decrease in one of the important secondary substrates, which is believed to be pathogenic Lyso-GL1 in individuals, both in plasma, as well as in the CSF. And in addition, there was some evidence for some improvement in some of the neurological examinations that were conducted. In addition, there was evidence for favorable safety and tolerability, robust reduction in CSF sphingolipids, and ability to ameliorate some of the neurological manifestations of type three Gaucher disease. Along with stable systemic manifestations, which may have been attributable to both this product, as well as to the continued enzyme replacement therapy.

So continuing, however, with the idea that enzyme treatment has not perhaps exhausted its potential in Gaucher disease, I'd like to show in the next slide, some recent evidence published with regards to a modification of enzyme therapy, whereas instead of the free enzyme being administered, the enzyme is actually enclosed within nano vesicles and infused for treatment of the chain disease. This particular preparation is not dependent on a mannose receptor pathway, and actually the enzyme activity delivered by this preparation was loaded to be higher in glucocerebrosidase deficient cells, than as was observed with any of the comparable enzyme replacement products, which were studied as controls. In addition, this nano vesical preparation was able to penetrate the blood brain barrier into the CNS. This CNS targeting was mediated by surface phosphatidytserine in the blood vessels and in brain cells. And in fact, increased glucocerebrosidase activity, and reduced glucocerebrosidase substrate levels were found in the mice models with Gaucher disease, which also showed evidence of profound improvement in brain inflammation and in the neurological phenotypes.

So again, this is an example of what is also taking place in other lysosomal storage diseases, namely attempts to continue to improve on existent enzyme replacement therapy. So I'd like to turn now to a different storage disease Fabry disease, which is one of the X-linked lysosomal storage disorders. In which heterozygous women are often symptomatic as well, sometimes as severely as their male relatives. Multiple generations are oftentimes infected with extended family groups. Pathogenic variants in the GLA gene that encodes for the lysosomal enzyme a-glactosidase are responsible for this particular LSD. And the deficiency a-glactosidase activity leads to failure to catabolize the various glycosphingolipids, but most prominently the one which is known as GL-3 or globotriaosylceramide. GL-3 accumulates with the lysosomes multiple cell types in vital organs and leads directly or indirectly to cellular function, microvasculopathy, end stage tissue damage, patient suffering and shortened life expectancy. In the classic phenotype, which is associated with a number of different GLA mutations,

Patients suffer from multisystemic involvement. Including manifestations, such as paraesthesia within the arms and legs, neuropathic pain, certain crises, which can be associated with marked exacerbation of pain, as well as febrile episodes. Episodes of crises associated with abdominal pain, episodic diarrhea, nausea, vomiting and weight loss. Patients with the classic manifestations often develop skin lesions known as angiokeratomas. And can have problems with sweating, heat intolerance, Corneal opacities, as well as hearing loss. And the life shortening complications include renal failure, cardiac complications and episodes of strokes, or white matter deterioration within the brain. Additional manifestations include chronic fatigue, oftentimes growth retardation, delayed purity impaired fertility. Changes in the joints and bones, osteopenia, chronic or bronchitis, and ultimately impaired social functioning associated with depression and decreased quality of life. Enzyme replacement therapy exists in two forms in the United States, agalsidase-beta. Otherwise known as Fabrazyme. Has been approved in other parts of the world agalsidase-beta and a second product agalsidase-alpha are used as well.

Both products seem to be basically similar. The one major difference seems to be that the agalsidase-alpha is usually prescribed in a dose one fifth, that in terms of milligrams per kilogram than is usually prescribed for agalsidase-beta. But why that enzyme should be more effective in a lower dose, continues to be somewhat unclear. The therapeutic responses are similar for both enzyme products, despite the different dose levels. Both enzyme products in treated male individuals decrease plasma GL-3 levels, as well as urinary GL-3 levels. And have been shown to decrease deposition of the substrate in kidney epithelial cells, as well as in various other cells within the kidney itself. There is some evidence that these products may reduce or stabilize left ventricular mass and thickness in some individuals, and improve perhaps some of the auditory symptoms, as some of the GI symptoms and some of the neuropathic symptoms.

Similarly, they can improve the clinical manifestations in affected women as well. However, again, as was the case with Gaucher disease, enzyme replacement therapy for Fabry disease has not proven to certainly be a cure. At best, it seems to slow the inevitable progression of the disease, and it may very well be however, that that is a factor of the patients starting enzyme replacement therapy. Usually after they already have evidence of irreversible organ damage in the kidneys, in the brain and in the heart. And perhaps where enzyme treatment started very early in life. And this is still somewhat of an open question. It is possible that the responses might be better than has been observed when treatment has started much later after patients are overly symptomatic.

There is a third approved treatment, a so-called chaperone therapy, which is a small molecule treatment, which is effective only in individuals with certain types of mutations, which are called amenable mutations, because this chaperone therapy works by stabilizing the actual configuration of the alpha galactosiadase within the endo plasma particular. Enabling it to be transported successfully to the lysosome where it is stabilized, and where it can actually be effective in catabolizing the breakdown of a GB-3. In studies, the imigalistat product has been shown to be effective for patients with these amenable mutations.

But again, it is not totally clear that even though it will retard progression of the disease, it may not prevent and totally stabilize, and prevent patients from still developing end stage renal disease as well as cardiac disease. So, some of the limits of enzyme replacement therapy, which were recognized in Fabry disease back in 2010, included that prevention of ultimate development congestive heart failure had not yet been demonstrated. And I believe that still continues to be the case. There was little evidence of benefit regarding cardiac arrhythmias, which led to complications in individuals with Fabry disease. No effect on prevention of ischemic stroke. And unfortunately, unlike Gaucher disease, patients with Fabry disease frequently developed cross reacting antibodies to the various ERT products, which probably altered tissue distribution, and may have limited the response to both the agalsidase-alpha as well as agalsidase-beta/. In terms of newer developments, there currently is study of a substrate restriction therapy known as lucerastat, which is a also inhibitor of glucosylceramide synthase.

The reason this product may be effective in Fabry diseases is because, ultimately, by preventing the first step in the synthesis of the glycosphinglipid, it's hope that there would be limitation of the amount of the later synthesized lactosylceramide and globotriaosylcereamide GB-3 product. And in that way, again, allow the effective enzymes in Fabry disease to work more effectively provided that the substrate amount was limited. In some of the early trial results were reported back in 2020, there was just some very early data suggesting that the product did not have any relevant safety abnormalities in reports from the six month randomized placebo controlled trial. There was actually very little reported in the way of endpoints in the patient's treated. And that this particular point I must confess, I have not seen much further in the way of information beyond the six month trial period, at which point there had been some reduction in the pain scores, measured with the brief pain inventory tool. As well as in some decrease in abdominal pain score as well.

And some decrease in plasma GB-3, Lyso GB-3 and some stabilization in renal function. But beyond this report, as I say so far, I have not seen any published follow up since that period of time. Again, as I mentioned with regards to Gaucher disease, there was also some attempt currently looking at whether enzyme replacement therapy can be improved for Fabry disease as well. And in this case, the enzyme under study is a alpha-galactosidase, which is synthesized within a plant cell bioreactor. And the enzyme is then PEGylated, hoping that this will provide a more stable product within the plasma, thereby enabling longer sustained plasma concentrations and more favorable fibro pharmacodynamics. In reports of the current study, which have been published to date recently in the journal of inherited

metabolic diseases. There was some evidence suggesting that the pharmacodynamics were indeed improved at various dose levels.

And there was evidence for decrease in substrate deposition within the kidney peritubular capillaries following, the six months of therapy with this enzyme product, which as you can see was a stable dimer with the use of the PEGylation. Recently, it was also demonstrated that the PEGylated product was able to lower affinity for potentially cross reacting anti-drug antibodies, which existed in patients who have already been treated with the standard anti replacement therapies. And therefore the conclusion of this recent study, published just in July of 2022, suggested that because of this potential for decreasing the affinity for anti-drug antibodies, that this product might be a promising therapeutic option for the majority of patients with preexisting anti-drug antibodies.

And it was recommended that patients should be tested before switching to this product in order to better predict therapeutic success. And a therapeutic switch is likely to be most effective when irreversible organ damage, including fibrosis, has not yet occurred. Turning from Fabry disease in the next slide to the Pompe disease or deficiency of acid alpha glucosidase activity. Pompe disease exists in two major phenotypes. The infantile onset Pompe disease, which is a rapidly progressive disorder associated with hypertrophic cardiomyopathy, generalized muscle weakness, loss of independent ventilation and clinical deterioration of affected infants with inability to roll over, sit or stand in early death from heart and respiratory disease.

The second major phenotype of Pompe disease is a so-called late onset. A late onset Pompe disease, in which symptoms develop usually any time after 12 months of age. The initial signs are associated with enzyme, with exercise intolerance, muscle pain and the severe muscle fatigue. The limitations of the previously approved enzyme replacement therapy for Pompe pain disease include persistent muscle weakness. Despite the enzyme replacement therapy patients continue to have a characteristic gate disturbance, lumbar hyperlordosis, progressive scoliosis, and they combined neuromuscular respiratory dysfunction, again requiring chronic assisted ventilation. Many patients also go on to developing swallowing difficulties, gastro esophageal, right reflux and aspiration. And this may be attributable to excess of the glycogen within the brain, with associated white matter changes again, because enzyme therapy does not cross the blood brain barrier. These later effects proceed despite some of the limited successes associated with enzyme therapy, in terms of the skeletal muscle defects associated with the disease.

In addition, some of these patients also show inefficient glycogen clearage with smooth muscle, and they may, as a result, have a secondary decline in walking ability, muscle strength, and a pulmonary function. There also after initiation of ERT seems to be no correlation between symptom severity and muscle damage. And muscle biopsies often show piles of debris and undigested glycogen, in the muscle fibers, preventing contraction of the muscle fibers. And this appears not to improve with enzyme therapy as well. So as a result of the unmet needs of the previous enzyme replacement therapy, a second generation therapy was approved in 2021, known as avalglucosidase alfa, for patients with late onset Pompe disease.

This enzyme has actually been modified and designed for increased cellular uptake and improved glycogen clearance. In the initial trials, which led to approval of the product, 24 patients were enrolled, 10 of whom were naive, and 14 of whom had been switched from the previous enzyme therapy. Of these 24 patients, 17 have continued on the avalglucosidase product long term. And data has now been reported up to six and a half years of starting that therapy.

During this period of time, there were no deaths reported the treatment patients. And similarly, there were no treatment related life threatening serious adverse events. 18 participants did develop anti-drug antibodies, but apparently these did not have any significant impact on the clinical outcomes. In terms

of the outcomes with regards to forced vital capacity measurements, in the naive patients, there continued to be some slight deterioration over time, which also was noted in switch patients. The six minute walk time was stable for most patients. Although again, some slow deterioration continued to be noted. In patients who were younger than age 45 years of age, however improvements in the six minute walk time distances, who are actually improved in most patients who have been enrolled in both the naive, and within the switch groups. A recent editorial, which accompanied the report suggests that, it's not yet proven that the new enzyme will indeed hold up over time in terms of greater efficacy versus the earlier product. But at least there is perhaps some hope that, that will be the case. And further study definitely is required.

Next slide, with regards to mucopolysaccharidosis and enzyme replacement therapy. Despite the fact that the various mucopolysaccharidosis are different in terms of both their biochemistry. And to some extent in some of their manifestations, the results for enzyme replacement therapy are actually pretty similar for the various MPS syndromes. A clear therapeutic effect for those which are currently available, have been seen only for a few outcomes. Namely decrease in glycosaminoglycan concentrations and in organomegaly. But this may be of somewhat limited clinical significance. There has been some observation of clinically important reductions left ventricular mass index and in the hypertrophy of the septum, and improved cardiac ejection fractions. Also there have been some reports of the decreased cardiac valve of thickness, particularly when ERT has started very early in life, fewer episodes of structure of sleep apnea, and generally sustained improvements in test of endurance.

However, there are continued areas of less successful response and unmet need. Namely, corneal clouding and hearing loss seems not to be improved with ERT, macroglossia and adenoid tonsil enlargement also not improving. Deformities of the trachea, bronch eye and blood vessels, seem to persist. Growth and skeletal involvement may possibly be favorably impacted with early treatment, but may not be averted when started later. There is also some evidence for possible inhibitory effects of anti ERT antibodies, where just improved joint range of motion has been observed. It seems largely to have been confined to the shoulders and not to other large joints. And there is little evidence for successful addressing of CNS manifestations, but there are starting to be some ongoing interest in looking at possible combination treatment regimes for better outcomes in the future.

One investigative study that I'd like to discuss in the next slide is long term open label extension study of, Idursulfase-IT, given for patients with the neuropathic MPS variant, MPS-II. This therapy, unlike standard enzyme replacement therapy, is administered intrathecally. And here, there were some improvements which were loaded with the intrathecal therapy, particularly after 52 weeks. What was reported here was improvement in various functional scores, which suggested that some of these actually did show clinically meaningful improvement in the DAS to GCA scores, particularly in individuals who were younger than six years, when therapy was started. However, on a discouraging note, after many, many years of extensive review, and regulatory discussion, unfortunately the data were found to be insufficient. To meet the evidentiary standard, to support a regulatory filing. This IT therapy will continue to be made available to patients currently enrolled in the trial, but, and they will be allowed to continue on this until perhaps some better therapy subsequently becomes available.

The next slide I'd like to mention, again, some work with regards to the various Sanfilippo MPS types. These are four clinically indistinguishable lysosomal storage diseases, that all result in accumulation of Heparan sulfate. They are generally quite rare diseases. They're global incidents varies by geographical region. MPS-III A, is caused by a deficiency in an enzyme notice as N-sulfoglucosamine sulfohydrase. The disease manifestations of this particular type, usually start between one to two years of age. The CNS is affected, gray matter more so than white matter. And unfortunately, most children usually die before age 18. And so far, at least no effective approved treatment has been available. So for patients for this

particular condition, again, there's a look at a chemically modified enzyme replacement therapy and results from an open label non-controlled multicenter trial, have been reported.

Six patients age once to six years with a developmental age greater than 12 months have been treated with weekly IV injections. Again, anti-drug antibody development was common, but in the patients, there was a traversal of the blood brain barrier, and improved pharmacological activity on the heparan sulfate in the CSF. Also, neurocognitive development, age equivalent score stabilized for all the patients who have been treated, but so far, at least no clear overall clinical effect has been observed on adaptive behavior or the sleep pattern or quality of life.

So in conclusion, I'd like to finish and return to a Gaucher disease, and where there is perhaps evidence that progress in treatment developments will continue to be the case. And I think that if one looks back to the early start of enzyme replacement therapy, which did result in Dr. Brady being awarded the medal of freedom, for his efforts as a pioneer in this development. I think it's fair to say we've come a long way. I think there'll be a light of a lot of exciting developments in the future. And this area certainly remains as one for fascinating opportunities for future investigation.

Thank you very much.