Özlem Göker-Alpan :

Hello everyone. I would like to welcome everyone to the CME series on lysosomal disorders. This is the fourth season for this very after-sought event, and I am the host of this series, Özlem Göker-Alpan. Today, we are going to be talking about the brain and lysosomal disorders. This is not the first time we are discussing this topic, and why we are coming over and over, simply because as many refer to the brain as the most vulnerable and least forgiving organ, is invariably involved in almost all the lysosomal disorders. We can ask the question, why does lysosomal dysfunction is a primary impact on the nervous system and why we are seeing nervous system involvement when there is lysosomal dysfunction? The reason is the neurons are highly dependent on the lysosomal system because they are long-lived and they're the post-mitotic cells, and they have highly extended axonal processes.

Let's ask the question in a reverse, why lysosomes are essential for neuronal health. Because the post-mitotic neurons require lysosomes for normal cell function, which basically requires getting rid of the intracellular waste to prevent the accumulation of damaged refolded proteins. This is also how the cell death is regulated through autophagy and other cell death pathways the lysosomes are directly involved with. In addition, there is the mitochondrial involvement and we know that the energy depletion is a major impact on the lysosomes too. And when we collectively look at the more than 50% recognized type of the lysosomal disorders, basically that sphingolipidosis are the main patient population we see. But in all the other conditions, actually that is nervous system is involved one way or another, including even the disorders such as Pompe disease in the late onset, even though it's a major muscle disease. But we know that with the increasing evidence, the CNS is also involved with strokes and so on and so forth.

So this pie chart represents the percentage of the lysosomal disorders that comes to the clinic with brain involvement. The proportions are just for illustrative, so it's not really exact depicting the percentage. But the majority of the conditions that come with a CNS presentation is the neuronal ceroid lipofuscinoses, leukodystrophies, and also the NPS, the neuronal types. And also we have the Fabry disease, where there is the strokes and the white matter involvement also can be another form of CNS involvement. So when we look at the disease spectrum in the lysosomal disorders with the neuronal involvement or CNS involvement, the NPS is with the attenuated forms, or we call them non-neuropathic, but some of them may have some mild manifestations of neuronal involvement. And then we have the Tay-Sachs, the late-onset forms, and then the Gaucher disease, Fabry, and Pompe diseases on the other side.

In most disorders, there is a juvenile and infantile form, and then in some neurodegenerative process begins in utero such as NPS types 4, 7, and Gaucher type 2. These are our learning objectives today and I would like to invite our speaker, Dr. Raphael Schiffmann, Raffi Schiffmann, who is a colleague, friend, and mentor for over 20 years. Dr. Schiffmann is an international expert in lysosomal storage and other rare neurodegenerative diseases. Trained as a neurologist, he often refers himself as the research neurologist because he has decades of experience advancing both clinical and translational and also drug development research. He has done pioneering studies on the neurological manifestations of multiple lysosomal disorders, and that shaped the field's understanding of disease mechanisms, treatment approaches, biomarkers, and the list is very wide and long. And so without much actually that's spending time on Rafi's experiences, I would like to give the word to him and then for this wonderful presentation that we're going to have for the next hour.

Dr. Raphael Schiffmann:

Thank you so much, Özlem, for this generous introduction, and your introduction to my talk is really excellent. Hello everybody. These are my disclosures. I'm basically a consultant for a number of biotechnology companies in the field. The whole concept of lysosomal diseases is that they are caused

by errors in a gene that produces enzymes that take out and recycle the rubbish. If the recycling enzyme is missing or deficient, the rubbish or garbage accumulates and builds up in lysosomal cells to toxic levels. You can see here on the left-hand side, a normal neuron, and to its right, a neuron chock-full of lysosomal storage. So the idea was that it's like in your kitchen when you don't throw away the garbage, it accumulates and obviously you cannot cook and work there when there's so much stuff there. The reality of course, is much more interesting and complex. Here is the pathogenic cascade. Of course, on top is the gene defect, the protein defect as a result, and then there is substrate accumulation, which causes a complex downstream pathogenic cascade.

Then we are trying to understand and it's not easy to figure out, which leads to cell dysfunction and death on many occasion, and of course, symptomatic disease. So a few words about the mechanisms of disease in lysosomal conditions, what can divide them into non-specific mechanisms and specific ones. Let's show example of a non-specific mechanism. You can see on the left-hand side that a description of autophagy. A defect in autophagy is the most commonly-cited non-specific mechanism in the majority of lysosomal diseases. Here is depicted Pompe disease, Gaucher disease, Niemann-Pick type C, mucopolysaccharidosis and free sialic acid storage disease, as an example. This is a process where the cell recycles its components and it consists of a vesicle that takes up the cargo, matures, and eventually fuses with a lysosome, and the catabolic enzyme in the lysosome break down the material, which can be membrane or organelles and digested to its small molecules, which then are recycled. This causes mitochondrial dysfunction or inflammation, oxidative stress, and in many cases can lead to cell death by apoptosis, for example.

On the right-hand side it's the same idea, but here the author emphasizes the secondary accumulation of glycosphingolipid because the primary substrate, when it accumulates, can inhibit other catabolic enzymes. And that leads to secondary storage and defective autophagosome-lysosome fusion, which as I discussed, leads to tertiary storage and cell damage and dysfunction and death. Let's talk about an example of a specific mechanism. There are very few specific mechanisms that have been described and identified in lysosomal diseases. One of them, which I particularly like, is GM1 gangliosidosis, which is in general beta-galactosidase deficiency. It is a combination of a disease of the brain and of the skeleton and can present at different ages. And the earlier, the more severe it is. It's basically a combination of skeletal dysplasia and brain disease. So investigator checked the brain of models and of humans under the electron microscope and they found an increase formation of contact sides between the endoplasmic reticulum, or ER, and the mitochondria here in the middle and the ER on both sides. And these are the contact sides.

They investigated that and their conclusion was that the GM1 gangliosidosis does not only accumulate in lysosome, but also in the endoplasmic reticulum, the ER. The accumulation of this glycosphingolipid promotes the formation of pores between that link, the ER and the mitochondria, and less accessible amount of ionized calcium in to the mitochondria, which leads to apoptosis. This is a very nice specific mechanism that has been discovered. Let's switch now to the neurological manifestations of these diseases. And basically, anything that can go wrong with the brain can occur in lysosomal disease. This includes developmental delay and cognitive regression, seizures, myoclonus, hypertonia, evolving to spasticity and mixed tone abnormalities, cerebellar ataxia, and gait disturbance. Extrapyramidal signs, psychiatric behavioral abnormalities are common, and loss of vision. Now, it's not only the CNS that is involved. The peripheral nervous system can be involved. For example, in Fabry disease and small fiber neuropathy, and in the local dystrophies there is a demyelinatic neuropathy of larger fibers.

In addition, as you heard already from Dr. Göker-Alpan, for example, cataplexy is quite specific for pneumopexy, Fabry disease has a propensity for ischemic stroke, and there's a lot of neuromorphological signs and abnormalities, both of the retina and the movements of the eye, which I

will emphasize. Sensory neural hearing loss, sleep disturbance, autonomic dysfunction is seen in particular in Fabry disease in the chronic form of GM2 gangliosidoses. Now, last but not least is bulbar dysfunction, which is really critical because it leads to dysphagia and aspiration, which of course leads to a progressive or acute respiratory insufficiency and is the major cause of shortening the life of these patients.

These diseases of course, present in childhood, infancy, and childhood and as Dr. Göker-Alpan emphasized, they include in particular Krabbe disease and metachromatic leukodystrophy, both leukodystrophies, Niemann-Pick type C, or NPC, GM1 and GM2 gangliosidosis, and of course the neuropathic mucopolysaccharidosis. However, this can present in adults, and this is very important to realize because it's very often unidentified and lead to a delay in diagnosis. What I'd like to emphasize here is this psychiatric and behavioral changes that can occur primarily before other abnormalities. There is even a form of metachromatic leukodystrophy that does not have any other dysfunction, only behavioral and cognitive changes. Similarly, in Niemann-Pick C, we know we identify only a third of the patients that we expect, and I think the majority are hiding in psychiatric wards because of the abnormalities at cognitive and psychiatric abnormalities.

That onset, the GM2 gangliosidoses, is important to be aware that it can present as lower motor neurone disease that very much looks like spinal muscular atrophy or ALS. So when you have a picture like this, always check for GM2 gangliosidosis, and of course they develop later cerebellar ataxia and other abnormalities. And of course, you can see cerebellar atrophy on the MRI. Fabry disease, neuropathic pain due to small fiber neuropathy, deficiency perception of cold and more propensity for stroke and vestibular cochlear involvement. And finally, Gaucher type 3 can present in adulthood, either extension from childhood into adulthood or new onset in adulthood with supranuclear loss, horizontal saccades, ataxias, seizures, cognitive abnormalities, etc.

So how to evaluate and follow neurological involvement in a patient with lysosomal disease. It's very important to perform a detailed history, including family history of course, any genetic disorder and a good general examination and comprehensive neurological examination. In a neurological examination you cannot skip really anything. You have to look at the mental status and development depending on the age, motor and cognitive skills, social-emotional abilities, and of course, observe their speech and thought processes. The cranial nerves are critical, in particular examination of the movement of the eyes. You have to know the difference between saccade and pursued eye movements. Check the hearing since hearing impairment is common. Motor function should be examined in detail, tendon reflexes checked from the jaw to the ankle. And plantar responses are important also because when they are going, but when you have a Babinski sign, it is an indication of the involvement of the pyramidal nervous system.

Sensory function is important, but it's very hard to assess reliably in children and individuals with poor cognition. Coordination and balance should be checked, and of course, the gait spasticity and weakness can be present. I strongly recommend, and this is what I do every time I see someone with such a disease, I record by video their examination, especially the gait, and this way I can assess it over the years in a precise way.

Examples of neurological and other findings. Of course, you have to look at the neurology in Fabry disease, but also don't forget the skin beyond your keratomas in the skin that is seen pretty exclusively in classic males. In Niemann-Pick C, exactly again, the vertical eye movement abnormalities should be looked for, dystonia, ataxia, and cognitive impairment. Gaucher disease, instead of vertical, it's horizontal supranuclear gaze palsy and the other abnormalities I described. Reflexes changes are common in metachromatic leukodystrophy, and importantly, in mucopolysaccharidosis you should be

aware that in addition, there is a propensity to develop hydrocephalus in particular in MPS 1 children, and spinal compression and other skeletal abnormalities that can be significant.

What tools can we use to follow up individuals with lysosomal diseases? Neuroimaging is a very useful one that is unfortunately underutilized. Here in the example on the left-hand side, GM1 and GM2 gangliosidosis measurement of the volume of the basal ganglia and white matter. The volume in general, the volume is the most commonly assessed parameter in neuroimaging and can be measured extremely precisely. You can see that the infantile form progresses faster than the juvenile form and you can compare with the normal control that they have. Very, very nice here. And similarly in the white matter, you can see the differences in the volume of the white matter in the patients compared to control. Another nice study on the-hand side, CLN3, where they study repeatedly and showed a decline, a qualitative decline in the cortex of volume, white matter, cerebellum and other areas in the brain. And of course, when you have that, when have a decline in the volume of the brain, you always have an increase in the size of the lateral ventricles due to atrophy.

You can also score the MRI here cross-sectionally. They scored in the infantile form and differences of course, in the progression between the infantile and the juvenile, or you can follow individual subjects longitudinally and you can see the difference. The bottom line is that there's often a correlation between the changes in the volume and the behavioral and neurological abnormalities, which validate the usefulness and the importance of these assessments. And here you can see in a number of areas in the brain, the significant correlation between the volume and the Vineland. We studied a number of years ago, individuals with Gaucher type 3. They were all adults on substance reduction therapy and what we saw, the blue diamonds are individuals who had good drug exposures. So you can see they tended to have actually an increase in the volume of the brain over one year. By chance we had one participant who did not have any drug exposure after the first few weeks. And this individual shows largely atrophy, which again, in combination with enlargements of the ventricles.

The biomarkers, you can measure a number of things in the blood and the CSF. Here is an example of neurofilament light chain, which has been very useful in neurology in general and has been used in many diseases. On the left-hand side, Pompe disease, you can see over the years that their neurofilament light chain, or NFL, increases with age while the control basically decreases. And that's really it shows the critical importance of measuring the patients and with controls of a similar age. On the right-hand side is work by the Göker-Alpan group in Gaucher disease, again looking at neurofilament light chain, what they show is that in Gaucher type 1, that there's no primary neurological involvement in the patients. No one has elevated an NFL, neurofilament light chain, in the blood. But while those with the acute form, the most severe form of Gaucher type 2, everyone has elevated NFL. And as expected in the more chronic form, Gaucher type 3, some have elevation of NFL and some do not.

I really hope that this is going to be used as one of the important indicators of clinical efficacy in a trial and also in clinical practice. Of course, it's very important to have cognitive and second order tools to follow the patients, and we divide them by non-specific ones and disease-specific ones. How do we monitor them, first of all? We use standardized developmentally appropriate neurocognitive batteries at baseline and regular intervals depending on the age of the patient. The nonspecific ones include the R bands, the valent, the Bailey and differential ability scales. These are quite well-known. Importantly, there are a few disease-specific scales that have been used and actually were found to be quite useful in clinical trials. There are very few, basically three plus a few in Fabry disease, which is particularly people use disease patient reported outcome, or ERO. For example, there was one that was recently developed by Sanofi. Unfortunately, it's not freely available. Additional testing recommendations for neuropathic lysosomal diseases in individuals with sensory, motor, behavioral and systemic comorbidities. I

recommend this article which describes the importance of taking all this, the other abnormalities that the patients have into account.

As I mentioned, restricted access to some of these tools because they're proprietary is a problem. What I use often is of course, emphasis on the interviews and I follow the medication use, which can sometimes be quite revealing. So you can depict the trajectory of this works only in children, and here you can express the developmental age that you assess in a variety of ways with the chronological age. And normally in this gray line, of course they're clearly correlated and should be in theory identical. But what you can see here quite nicely is in this individual, gross motor lags the most behind the correct developmental abilities, while receptive language follows the normal developmental progression quite closely. You can use this to assess individuals, but also to assess the natural history, but also response to a variety of treatments. Again, I do emphasize hearing loss, visual impairment, sleep difficulties, cardiac abnormalities and respiratory ones, joint deformities and swelling as additional medical complications that should be taken into account when you evaluate such patients.

Here is an example of CLN2 rating scale, which was used actually to get a drug approved. I like it because of its simplicity also. There's a wide Cornell version and the Hamburg one. And for example, if you look at gait, it's very easy to assess normal from abnormal but independent gait, abnormal requiring assistance, and non-ambulatory. So the simplicity of this is quite useful. Here is the Niemann-Pick C. This severity scale was constructed and developed by the group at the NIH. It has 17 domains. This number of course, is quite unyielding in clinical trials, so in practice what people have been using is the domains of ambulation, swallowing, speech, fine motor and cognition. So the five domains are being used. But in fact, the FDA actually preferred to use only four for their analysis and they dropped the fine motor skills. And they claim that if you do that, you get less noisy results that are better than the five domains.

How you apply these? Here's an example of a drug called N-acetyl-L-leucine in a randomized blinded control trial, a placebo control trial. You can see that those that were on the drug progressed more slowly than those that were on the placebo over one year. That's a quite reliable and clear result. When you don't have a control group such as that, then you use, for example in registry and open label studies, you use the natural history. If it's high quality and quantitative, you can show here that over a year on this drug N-acetyl-L-leucine, there was only 20% of the progression expected if you look at the natural history in untreated individuals. So it's less reliable and convincing of course, than a randomized control trial, but it can be also quite useful.

How do we monitor? It's important to integrate multi-domain data on cognition, adaptive behavior, executive behavioral symptoms, but also includes sleep and seizure burden and the burden for the caregiver. You have to recognize that the behavioral and executive dysfunction can be prominent, even individuals with global IQ that's near normal, which is seen especially in attenuated forms. You have to corroborate these data with what you see in neurological and ancillary biomarker, and for example, but it has to have relevance to cognition. So of course, neurological examination, the brain MRI, and the biomarkers in blood and CSF, as I gave you example of. Timing of the assessment is very important. Of course, with pre-treatment, at baseline, and a frequent post-treatment or on-treatment follow-up using the same instruments to detect treatment-associated changes.

It should be a team-based execution in partnership with the caregiver using quiet, sensory-adaptive setting, augmentative communication when needed, and scheduling shorter repeated sessions to optimize the validity in patients with hearing loss, vision impairment, and other such abnormalities. When you are studying groups, it's important to analyze things centrally. Be careful to make sure that there is rated training, use of translated and validated version for international cohorts, documentation of the conditions of the testing, and proactive planning for transitions to alternative measures as

abilities decline to maintain longitudinal continuity. That's really important. When a subject declines or gets even older, you have to adjust the tool that you use to evaluate the person.

My last slide is a discussion on trends in clinical research to improve the outcome in patients with lysosomal diseases. The first one is autologous lentiviral hematopoietic stem cell gene therapy when you take out the individual's stem cells, you capture them in the lab. Transduce the healthy gene using a lentiviral vector and then reinjecting into the recipient into the patient. There's some interesting results of this approach in MPS1 and potentially it is approvable. However, the majority of gene therapy studies use adeno-associated virus, or AAV, in a number of lysosomal diseases. The problem is that you have to screen for individuals who do not have antibodies against your capsid and you can lose about half the patients like that in some cases. And after you administer the gene therapy once, everybody develops antibodies against the capsid, so you cannot with current technology re-administer or re-dose.

Also, there's a question about the durability of this treatment over the years, and in some situations there were immunological complications that can be sometimes severe. So there's no question that the future is gene editing, where you correct the mutation, the primary cause of the disease genetically. And this is the future of course, but the challenge is to make sure that the delivery of vector arrives into almost every cell in the brain. So deliveries has to be worked on, and of course efficiency, as well as making sure that there's no off-target effects. But I think that's the future and has been tried in other diseases. You may have heard recently in Huntington disease and others. Freidriech’s ataxia is another. Enzyme replacement is a therapy engineered for the CNS. Injection of the enzyme into this spinal fluid of the brain remains proof of concept. This product, cerliponase alfa, has been approved, by the way, using the scale that I showed you and is being used in this form of NCL.

There are other ways to improve the injection and the methods of delivering enzyme into the ventricle, into the CSF, and this is being developed and showing some promise. However, small molecules are still going very strong, in particular in these diseases. Substrate reduction and pharmacological chaperones, these are molecules that are specific and they penetrate and cross the barrier into the brain. Repurposing of all drug has been quite successful recently. N-acetyl leucine is a version of a drug that has been used since the 1950s to treat vertigo, has shown improved ataxia in a three-month study with favorable safety profile, and the long-term disease looks quite promising in terms of stabilization. The other molecule is arimoclomol, was approved in combination with miglustat to stabilize the disease long-term, and I show you some of the result. However, there's an increasing use of sophisticated artificial intelligence to solve structures of proteins and enzymes, and design rational small molecules that either activate or inhibit the enzyme or the protein. And this is really the future. They have, for example, people who develop non-inhibitory pharmacological chaperone to a variety of lysosomal diseases.

Delivery innovation. Here it's also some exciting stuff, is the fusion of the therapeutic enzyme with a ligand or part of a ligand, it can be an antibody, towards a receptor on the surface of the vascular endothelial cell in the brain. And this way, you trick the receptor to let the whole enzyme into the brain. And that shows initial results that are promising, for example, in Huntington disease. For completeness, I mention focus ultrasound, breaking of the bloodstream barrier or opening it. It looks interesting in some cases but I don't think it's going to be widely used. There are optimized other ways to deliver drugs. And what's interesting recently is the engineering of enzymes that have increased stability and half-life. There's a very interesting example in models of Gaucher disease type 3 recently, where they showed using AAV, that if you use just the wild type enzyme you get very little therapeutic effect, if any.

But if you use, all else being equal, the form of the enzyme that is stable with a long half-life, you can practically almost cure the mouse. This is exciting. Especially if it's in combination with a ligand to get the drug into the brain, can be even used as a form of enzyme replacement infusion to correct the

nervous system. All these are active areas of research to increase the delivery of enzyme and the vectors into the brain. That's it. Thank you and I'll be happy to hear if you have some questions. Thank you very much.

Özlem Göker-Alpan :

I thank Dr. Schiffmann for this wonderful presentation. I am opening the presentation for discussion and question. There is one question from an attendee and I have few questions if we have time. They're asking whether, can you explain why intrathecal ERT seems to be so difficult to develop?

Dr. Raphael Schiffmann:

Yeah, for a number of reasons. First of all, it's invasive, so it's not something that can be done casually in your clinic. Secondary, one problem is that in some cases, such as in the case of glucocerebrosidase in Gaucher disease, the enzyme is very, very unstable in the presence of CSF, and basically it inactivates it really, really quickly. So you have to hope that you have a form of the enzyme that is not inactivated. And finally, even though we deliver it to the CSF, the actual delivery to all parts, to every neurons or brain cell is probably incomplete. So this is some of the reason, but I want to emphasize that even if the treatment is not so impressive initially, if initiated early in life, really either prior to developing any abnormalities or very early in the process, it can be quite effective.

Özlem Göker-Alpan :

Okay. I have actually a few other questions if there is no other question from the audience. So let's just continue with the treatment modalities, the future therapies and innovations. As you presented, there are small molecule therapies such as substrate reduction and the chaperones, brain penetrant enzyme therapies that are in works and it's going to be coming up. And also, more currently there is, I think more common, AAV-mediated gene replacement therapies. Given the approval is expected in any of those, which treatment modality you think is going to deliver the most rapid transformation?

Dr. Raphael Schiffmann:

I don't think one can be sure in general. One has to take one case at a time in order to assess the likelihood. It depends of the efficacy of the particular molecule and it's safety. For example, N-acetyl leucine looks very interesting. In general, if you have a small molecule that goes readily through the blood-brain barrier, it almost ensures a complete delivery to every cell. If you deliver it using AAV, there's always going to be areas which are not seeing any, unless there's a tremendous oversecretion and picking up untransduced cell. So all things being equal, if you have a small molecule, this is highly effective, but by the nature of the treatment, it's easier to access every cell in the brain.

Özlem Göker-Alpan :

Okay. I guess we're going to be talking about treatment. Dr. Gustavo Mageava, actually he comments on your excellent presentation, and also he's asking about your thoughts about the prenatal enzyme replacement therapy for early-onset lysosomal disorders and the potential efficacy of it.

Dr. Raphael Schiffmann:

Oh, prenatal.

Özlem Göker-Alpan :

Yes, exactly.

Dr. Raphael Schiffmann:

Yes, it's something that I did not mention that has been tried in Pompe disease, I think by the group in UCSF. Yes, but it sounds quite heroic. And I think it was also tried or in Gaucher type 2 cases or when you expect somebody. We don't have enough experience, I don't think, to reach a firm conclusion. I am not sure that in the long-term that it's going to be tremendously useful. Again, if you have a small molecule that you can use in the mother that will cross into the placenta, that can be a nice idea. For example, in Gaucher disease, if you have a chaperone that can activate the enzyme inside the uterus, that can be great. But in terms of using enzyme to inject prenatally, I don't think we have enough experience.

Özlem Göker-Alpan :

I have one more question about the treatment and then we can actually have one more question about the patient monitoring. Now there are multiple gene therapy trials for multiple conditions, so who are the candidate for a gene therapy trial for a neurological disorder? I'll put it this way, a little bit more explicit, are the infantile forms of these conditions are candidates for gene therapy?

Dr. Raphael Schiffmann:

In principle, yes. As a general response to a general question like that, yes. You should not limit it to a particular disease variant. And sure. But one has to do case by case and see. In the condition that you are the world expert Gaucher type 2, for example, the onset of the disease it's so early that it's prenatal we think. So I don't know if gene therapy intervention could help. But in principle, yes, anyone should be a candidate at any age if there is an efficacious treatment.

Özlem Göker-Alpan :

So this is the last question we're going to take for treatment, because I guess there is no end to it. I want to ask one monitoring question before we close. So Eden Gill is asking, could we expect any benefit from the gene therapy to or three years after the trial, like in early GMs, GM1 and GM2?

Dr. Raphael Schiffmann:

So the durability is a big question here. There is this study that was just a few weeks ago published, Florian Eichler I think is the first author, when they injected in two different places, two different ways to treat, I think it was GM2 gangliosidosis. But even there, there was a clear decline over the years in the efficacy. I think only genetic editing would be a permanent solution. All the AAVs, I think sooner or later will decline in effectiveness. The question is, how many years? And the problem, again, what do you do afterwards? How do you eliminate the problem of the antibodies after the first administration?

Özlem Göker-Alpan :

One last actually comment from Dr. Mageva also, who reminds us about IV use of ambroxal in expecting mothers. Actually, it is approved in Europe and other countries for premature... Actually, that's for the lung prematurity. I just want to comment obviously the dose might be much different, because we know the chaperone dose for ambroxal is quite different than the expectorant dose.

I have one question about the patient monitoring, or actually incorporating this into the clinical trials too. For quantitative monitoring, which modality you think is more fruitful, including neuroimaging,

biomarkers like neurofilament light chain, or other disease-specific biomarkers, or the standardized cognitive behavioral scale or the cognition? So which one would you use in your clinical practice and trials?

Dr. Raphael Schiffmann:

That depends on the disease itself. For example, in Niemann-Pick C, as you saw, it's a classic neurodegenerative disorder that there is a clear decline that can be seen in a year or two. There, it's a lot easier to assess using a clinical tool. They assess whether you slow the progression of the disease. That's the best way to do a study. However, the other end of the story is what you are tremendously familiar with, Gaucher type 3, where actually there it's so slowly progressing that we cannot use the progression as a way to assess. We have to look for improvement. Absolute improvement. And that is facilitated by the fact that a lot of the brain actually remains intact in these patients. So every disease, it's very important not to generalize, and to go from one disease to the other depending on its natural history and the modality that you have.

Now, that being said, neuroimaging is very sensitive and less noisy than pretty much any clinical tool that you would use. So it wasn't necessary in CLN2 or in Niemann-Pick C, for example, to show a clinical effect. But in cases that are very slowly progressive, for example, neuroimaging is more sensitive than any clinical aspect, as I think we showed in our initial venglustat study. This applies to other disorders also. So depending on the case, you use one method or another. The neurofilament light chain is too early to say how sensitive it is in assessing treatment effect.

Özlem Göker-Alpan :

Okay. Unfortunately, our time has run out, and I would like to thank Dr. Schiffmann again for this extremely helpful and wonderful presentation. We also thank too, the sponsors of this program that include Ultragenyx and Takeda. And while I close the session, I would like to invite everyone to our next one in December and then the speaker will be announced. Thank you very much. Have a great day.