New and Emerging Phenotypes in Lysosomal Storage Disorders

Ozlem Goker-Alpan:

Hello. I would like to welcome everyone to our third series in the CME on lysosomal disorders. On today's topic, we're going to cover new and emerging phenotypes in lysosomal storage disorders with novel and effective therapies. As an introduction, I would like to start with my disclosures. So this is a continuing education activity. Its managed and accredited by Affinity CE, in collaboration with the LDRTC. And commercial support for this activity was provided by Takeda, Cheisi, Ultragenyx Pharmaceuticals and Spark Therapeutics. So as an introduction, I would like to start with the cell biology of the lysosomal disorders. The reason is, I want to cover a little bit, the principles of treatment, and what we had achieved by treating lysosomal disorders. Obviously, the prototypes of these are Gaucher disease, Fabry disease and Pompe disease. As we had covered Gaucher and Fabry disease extensively in the previous discussions, I would like to focus on Pompe disease as an introduction today.

To start off, let's just review the therapeutic approaches in lysosomal storage disorders, related to the cell biology or cell pathology. So obviously, if the missing protein is an enzyme, the logical aspect is to replace the enzyme, which decreases the substrate. And the other alternative is, a substrate is produced too much trying to control the substrate production. And the innate enzyme would be able to actually reduce the amount of the substrate deposited. The third approach is related to the folding or the misfolding on the enzymes. And one needs to remember, lysosomal enzymes in general, they're the proteins which have very long half lives. Some have a shelf life of 72 hours, which actually makes it amenable to misfolding and all the naive or native state of lysosomal enzymes actually can be misfolded.

And so to prevent its misfolding, and to actually enhance the folding, we can use chaperones, which are the molecules that can attach the protein, that is not fully folded to its tertiary stage in the endoplasmic reticulum and help the molecule to escape other degradation processes within the cell, and move it to the lysosome. And obviously these are the cellular aspects of the therapeutic options. And then we can discuss, obviously the hematopoietic stem cell transplant plantation, like the for MPS I or gene therapy protocols have been coming in multiple numbers recently.

We're not going to be covering these, but we are going to cover what we had achieved with the therapeutic options available, especially with ERT and to a certain extent SRT for some disorders, and whether we are creating novel disorders or not.

So, if you need to individualize the treatment for lysosomal diseases, we start with the culprit, which is the diseas- causing mutation and the protein. So basically, this protein can aggregate. Either it can gain function or loose function, which is the ERT. The substrate accumulation can be addressed by SRT. And then the downstream effects such as immune dysfunction and inflammation, which are expected to be alleviated with these available therapeutic options, but truly they need to be addressed individually.

So currently, even though theoretically these are effective therapies, it is quite personalized. Meaning if we give one similar dose to a multitude of patients that share the same genotype, we may get different

therapeutic response and then with treatment, the end-product of the treatment may be different. So that is the main issue in the lysosomal disorders, which is phenotypic variability. And depending on the genetic background of each individual patient, in addition to the complex cellular pathology, we do have the molecular chaperones, that is innate to each individual patient. And speaking of innate, we have the innate immunity, and the immune responses could be different in each patient, that will lead the inflammatory response and the chronic downstream effects of the disease process. The other major obstacle in curing or achieving therapeutic results, always a key to cure, is obviously the challenge to deliver the therapeutic product for enzyme replacement therapists. These are biologics, and these are very large molecules, and CNS is not accessible to these molecules in general.

And also we have issues delivering in bone and joints and lungs and other tissues that have different tissue accessibility to both the biologics and also similar to other small molecules, such as SRT. So obviously there is a repetition. I need to address the inflammatory and immune competent of the disease. All the LSDs are inflammatory disorders, and that may need to be addressed individually as the inflammatory and chronic response may be different from one patient to another. So I'm going to continue this introduction, presenting case reports on Pompe disease. Pompe disease, obviously the prototype of the changing landscape of the patient phenotype with treatment. Now, back in 1995, prior to ERT, these babies were doomed to die without therapy.

Obviously we provide quite effective therapy and we save them from dying due to congestive heart failure, but are we creating another disorder by treating these infants? And what do we need to address as our therapeutic aims in these babies? So, as an example, this is a prototype Pompe disease patient born in 1995. Typically, they present around four to six months of age. So, similar, this patient presented at four months of age with hypotonia, with a large tongue and congestive heart failure. If you see this kind of a baby in the emergency room, by then you would get an x-ray and this heart will fill basically the whole chest. So diagnosed on Pompe disease on the basis of presentation, but also the lack of acid alpha glucosidase in the leukocytes. And this baby, without actually intervening with a disease specific therapy, unfortunately died at seven months of age due to intractable heart failure. As opposed to the next patient that I'm going to present now, is born at 2015, when the newborn screening was available and was diagnosed with newborn screening.

And the baby was fine initially at birth, sent home at 36 hours after birth, however, became symptomatic the next day. And this is day three, two days. The newborn screening, on day five, was positive for Pompe disease or the zero enzymatic activity for alpha glucosidase. And the initial CK, which actually correlates with the muscle damage, was about 4,000, so normal CK is about 136. So this patient, after immune modulation, started on treatment, and he's in a playground at around age four. Is a normal kid that can keep up with their peers. So the ERT infusion was given at 10 days of age, he was hospitalized in ICU for six weeks to find the optimal treatment and also to be evaluated for sleep disorder, and then feeding issues. By age six months on therapy, he was able to transition from two feeds, and at 14 months the PE findings and all tests were all age appropriate and discharged from all other supportive therapies.

So at age four months, he's developmentally appropriate. However, not all the patients may be as lucky as this patient, who is similar to his peers in both muscle strength and development. So this slide

actually depicts the numerous screening results. And it actually describes long term prognosis of an infantile Pompe disease patient diagnosed with newborn screening. And these patients were actually treated all from birth. So we are seeing a different phenotype of disease in all the patients with treatment. So similarly these patients, who had initially CK levels increase, the CK levels actually returned to lower levels, sometimes normal, with excellent morphology of muscle biopsies after six months of ERT. However, the weakness of the truncal muscles occurred even in very early treated patients. So despite this, both biochemical and pathological tissue response, still these patients would have motor abnormalities. And in addition to the motor abnormalities, what is more important, now we are seeing signs and symptoms that didn't exist before, because all essentially babies died by around six to seven months of age.

Now we are treating these patients and we are understanding the basic pathology of Pompe disease, that involves the neurologic involvement also. So there was in these patients, neurogenic involvement with glycogen accumulation in both the anterior horn of neurons of the cervical spine, and the brain stem neurons of patients impairing respiratory, swallowing, and speech functions. Now we know that these abnormalities in these vital functions is not only due to muscle weakness, but also the nervous system dysfunction due to accumulation of glycogen in both spinal cord and the brain stem. And in addition, what we are seeing in the spinal cord and brain stem, there is also brain dysmyelination and observed both in latent treated patients, but they also now is being seen in early treated patient, which means that the ERT could not access the CNS manifestations of this disorder. So currently in this cohort, the relationship being between MRI findings and where there would be cognitive issues in the future, was not clear.

So in addition to both the dysmyelination and bowel dysfunction, the speech disorder actually came into picture. So the flaccid dysarthria that can be attributable to myogenic or neurogenic involvement and glycogen storage in the muscles involving speech, may not be prevented by the early treatment. And similarly, the reduced sensitivity of the larynx and pharynx and delay swallowing in treated patients with IOPD. In this case, the brain stem dysfunction, which is likely caused by the glycogen accumulation in the motor neurons of the brain stem in the infantile onset Pompe disease. So basically infantile onset Pompe disease, even if it's treated in the very early phases. So the life is definitely salvaged. So this is not the same disorder. We are having these babies live, but we are having these babies live with another disorder, that requires different guidelines of monitoring and treatment. So from this introduction, I would like to introduce to the next speaker.

We are very lucky, Dr. Uma Ramaswami is joining us from London, UK. She's the consultant in inherited metabolic disorders, and clinical lead for the lysosomal disorders unit at the Royal Free Hospital, London. And Uma has a specific, special interest in clinical research relating to understanding of the natural history, and disease progression of inherited metabolic disorders. She also leads the transition services for young patients with inherited metabolic diseases. Uma is extensively published. She actually authors more than one hundred publications related to lysosomal storage disorder. For today's presentation, Uma will focus on another lysosomal storage diseases or group of that with MPS.

Uma Ramaswami

Thank you very much Ozlem and the LDRTC for inviting me today, to present at your quarterly series. And in the next 25 to 30 minutes, I would like to focus on mucopolysaccharidoses or MPS disorders. An example to describe the change in the natural history and the new phenotypes that are developing due to better treatments over the past two decades. So these are my disclosures. So at the conclusion of this activity, participants will be able to consider the following: recognize new phenotypes with existing disease modifying therapy, such as enzyme replacement therapy. And I'd be focusing on MPS. I would also hope that some of the unmet needs and the value of multidisciplinary management in MPS will be addressed in this talk. And I will also briefly touch upon the emerging treatments and the exciting opportunities we have currently in the management of MPS, beyond enzyme replacement therapy.

So what has got better with enzyme replacement? Well, certainly the systemic symptoms such as reduction in liver and spleen size, there has been a reduction in the biomarkers, such as urinary GAGs, some improvement in joint range of movement and mobility, and indeed some improvement in endurance, as children. In childhood, there has been improvements in growth, and also an improvement in general well being and quality of life. And certainly the number of ear infections and chest infections had reduced in these children who are on enzyme replacement therapy.

However, not everything has got better, and there has been a significant inability to target all tissues equally. And I've just put a list here of some of the lysosomal disorders, but in particular, if we look at MPS I and MPS II, for example, the problems we have currently is that enzyme replacement therapy does not adequately target the bone, the brain, or indeed cartilage with ongoing cardiac valve disease, which is often asymptomatic in childhood, but as they survive into adulthood, this is now becoming an increasing challenge. And given the complexity of their airways and their skeletal abnormalities, and also short stature, anesthetic and surgical procedures of these young people within an adult setting has indeed been quite challenging.

And the ongoing cardiac problems, as I've already mentioned is symptomatic cardiac valve disease, and also arrhythmias in adults possibly causing sudden death in some of these patients. This needs to be evaluated further as there has been no systematic studies in evaluating the role of arrhythmias in these patients. But certainly in my own experience, we've had at least two or three patients who we thought were well and had unexplained sudden death. And I wonder if this was secondary to cardiac arrhythmias. The ongoing neurological problems in these patients include the hyperactivity and obsessive compulsive disorders. And we are also seeing retinal degeneration, both in MPS I and MPS II, as they get older and progressive dementia is typically seen in those patients with MPS I. But again, I think as these young people are surviving, we will need to monitor these symptoms very carefully.

The major issue we currently have with many of our young adults with MPS, both I and II in particular, include the upper obstruction, the severe tracheobronchomalacia, which can be at multiple locations within the trachea, and the respiratory insufficiency. And we think the airway problems in MPS are exacerbated by the inadequate clearance of GAG deposits from the adenoids and tonsils, the pharyngeal wall deposits, and also the presence of the skeletal dysostosis that include mandibular dysplasia and the macroglossia in some patients. And the MPS deposits on the lower end of the larynx, particularly the arytenoids and the aryepiglottic folds, and also in the tracheal wall, causes a prolapse of the GAG deposits. There's an increased deposit and this causes prolapse of these substrate deposits into the

laryngeal airway, causing that vibratory cough and stridor. And I think if you do see a patient with vibratory cough, then an early assessment of the airway, particularly the lower airways as well, is important.

So many of you are familiar with some of the supportive interventions, but as they get older, we need to now start thinking about the other supportive interventions. And there's very limited experience in any of these interventions. And then of course, the general surgical and anesthetic consideration in MPS patients should also include the progressive cardiac valve disease that we're seeing. And I do wonder whether cardiac valve surgery can be performed earlier, although when we discussed this with the pediatricians, indeed there is always the risk benefit ratio of major cardiothoracic surgery. Patients, particularly the MPS I, can have temperomandibular joint stiffness. And I've mentioned some of the other problems that we encounter with our young adults. And as they get into the adult clinics, given the lack of experience, the lack of equipment for the size of some of these children, it does become a challenge.

So what are the factors that limit the treatment response? Well, one is the disease itself, the disease being treated, and there are probably secondary events and I will allude to that in the next few slides. And some aspects may be untreatable, or a point may be reached when a disease is untreatable. However, having said that, many of our patients are young patients who've been on treatment, who're doing relatively well, but do tend to have some of these very challenging problems with their airways and cardiac involvement. And treatment itself may not correct all elements. And there may also be immune problems, for example, antibodies to the enzyme replacement therapy. So factors that limit the treatment response. I think the typical analogy was lysosomal storage disorder and the direct effect of storage causing tissue damage and disease manifestations. Whereas I think the new vision of lysosomal disease pathophysiology suggests that there are secondary and tertiary events, including the activation of cellular pathways that significantly contribute to the ongoing tissue damage.

And of course there is a lot of interest in the literature in regards to the accumulation of the mucopolysaccharide storage causing lysosomal dysfunction, including aberrant signaling and activation of inflammation, oxidative stress, and impaired autophagy. So I think the old adage of restoring storage equilibrium as a sole therapeutic option, I think is no longer sufficient. And therefore, I think we do need to also consider alternative therapies, or adjunctive therapies, to help our patients. As I mentioned previously, the immunological responses include the therapeutic enzyme itself being potentially immunogenic. And this could be the production of antibodies, which may in fact have no impact at all on the clinical phenotype. They could develop hypersensitivity reactions that could be decreased by availability or in fact, decreased clinical efficacy, should there be inhibitor antibodies. However, in our experience, generally, the disease modifying therapies for LSDs have been fairly safe.

And many of us know that over 50% of patients with lysosomal disorders have neurological involvement. And if we look at the neuronal loss per se, it is a potentially vicious cycle. So you have neuro inflammation that is associated with lysosomal disorders. It may be initiated in part by the disruption of normal lysosomal function and accumulation of heterogeneous inclusions. So the lysosomal dysfunction triggers the activation or indeed the release of these danger associated molecular patterns or DAMPs. The DAMPs activate the surrounding glial cells, resulting in their proliferation, and it initiates an

inflammatory cascade or signaling cascade. And this results in the secretion of cytokines, chemokines, and harmful toxins. And this environment then further causes neuronal death and the loss of glial support, for example, and further DAMP release. And this vicious cycle is continued.

So the strategies for lysosomal disease therapies are several, and the lysosomal disorders, as we are aware, are due to mutations that cause the synthesis of mutated proteins and the defective activity of lysosomal function. So you have this intracellular lysosomal storage of substrates and triggers, that trigger secondary cellular abnormalities. And as you can see from this slide, there are several stages where we can intervene either from correction of the genetic information, for example, gene therapy and stop codon read through, and more recently, there has been a significant interest in the manipulation of autophagy. And in fact, also in the inhibition of DAMPs.

So this is just a summary slide to show you the existing and emerging adjunctive therapies for MPS disorders. This may not be exhaustive, but I think it has the majority of the key studies that are currently underway. So traditionally we have bone marrow transplant, hematopoietic stem cell transplant, and enzyme replacement therapies for some of our MPS disorders. But there are several other studies currently that are addressing, for example, gene therapy studies for both MPS I and II using adeno AAV virus and also Lentivirus. There is also a gene editing study, which is currently paused, but I suspect will start soon for both MPS I and MPS II. There has been also trials for adjuvant therapies with small molecules, for example, pentosan polysulfate, which is an anti-inflammatory and pro condragenic properties used in rheumatoid arthritis, which is now being studied in MPS I and also MPS VI. There's also been studies of fusion proteins, for example. And in fact, the GC pharma intracerebral ventricular ERT trial has been completed and has been approved in Japan, but obviously there are more clinical trials that will be underway in other centers. And there are also new generation intravenous enzyme replacement therapies currently being investigated. So there are several exciting new therapies that are really needed currently, as an unmet need, in MPS diseases.

So, in summary, enzyme replacement therapy for MPS I, II, IVA, and VI has certainly improved quality of life, although we have limited experience with ERT for MPS VII currently. ERT reduces the biomarkers such as urinary GAGs, there are systemic improvements, and even some improvements in an endurance and in growth. But as I've shown you from my own patients, the emerging complications as they get older, and they survive into adulthood, is particularly challenging with the complex airway disease, the sudden unexpected deaths, and also the ongoing cardiac valve disease. But having said that, as I've already alluded to, we have an exciting future with many new therapeutic options that could complement current disease modifying therapies for MPS diseases, in my view.

And disease modifying therapies are really only one part of multidisciplinary care. And these children need a whole range of specialists, from the metabolic specialist to the orthopedic surgeon, the neurosurgeon, the nephrologist, the cardiologist, et cetera. And the transition from pediatrics, in my view, should include a wide range of multidisciplinary teams to address these issues, early in the course of the transition and definitely before transfer of patients to the adult centers. So with that, I just want to say thank you, and I'd be happy to take questions.

Ozlem Goker-Alpan:

Uma, thank you as always for this wonderful presentation. We have a few questions in the Q&A box here, but I want to actually combine them together because one of them is asking whether we are changing the phenotypes with ERT. And the other question is, whether also this implies the Sly syndrome, or is it too early to tell? So you can start with a general statement and that you can apply to different disorders as we go.

Uma Ramaswami:

So I thank you Ozlem and thank you again for inviting me to this, your autumn series. I think absolutely, we are changing the phenotype of even with Fabry, many of the adults who perhaps would've succumbed to either renal or cardiac disease in their third and fourth, or fifth decades, are now surviving. And in fact, we very rarely see renal failure, for example. So the most common cause of death now would be cardiac involvement. And I think with the improved treatments they're surviving. So certainly we are seeing a different phenotype in regards to that. And also as they get older, I think you will also be seeing more of the neurological manifestations with the white matter lesions and the impact it has on memory, for example. So again, all of that is now the changing phenotype, but we certainly have improved that quality of life for sure.

Gaucher disease, I think Ozlem, you probably will have more of the experience in both, I think GD III and also GD I. And with the GD III patients, for example, as they get older and surviving, then you have more of the neurological complications that might arise with that. For example, executive dysfunction, problems with ataxia, et cetera. So I do think we are seeing a slightly different phenotype of what we did perhaps maybe two decades ago when we didn't have disease modifying treatments.

Ozlem Goker-Alpan:

Can you comment on Morquio or is it too early?

Uma Ramaswami:

Actually Morquio A syndrome, I think, in fact, it's interesting, because I think with Morquio, the sooner we start therapies, I do think that there has been an improvement in their endurance, for example. So we certainly have young adults who've done reasonably well with MPS IVA on enzyme replacement therapy. And I think the ones who do really well are those who already have all of their orthopedic interventions and the survival spine fusion done early. And these are children who are ambulant when they become adults. And we don't see so much of the cardiac valve involvement in MPS IV, as we are seeing with MPS I and II. MPS VI, I think it's very variable. It depends upon the severity of MPS VI as they get older. So the more severe phenotypes I'm not sure really do well. And there are certainly certain genotypes we know, that don't do very well in this particular cohort, but certainly the older patients who are very mild seem to be doing relatively well at the moment. Yeah.

Ozlem Goker-Alpan:

So let's discuss a little bit by experience with Gaucher and so I have the actual privilege following the patients that are originally treated with the original cohort from Roscoe Brady. So these are the seminal 12 patients that I follow. And I also follow the second or the third generation patients that were diagnosed actually with family screening or with a known family history. And then you see the patient who is the proband versus the ones that are found to be after the proband, but at the time of the diagnosis, without that significant clinical impact. So as you follow these patients through, you can always tell the patient who is the proband. Meaning the patient who comes into the medical attention with significant clinical presentation will remain more severe when you compare with the others. Okay.

So, there are multiple reasons why you may have that. And then we actually look into a group of patients who underwent splenectomy, and then we compared the ones who were not splenectomized and then we actually did a little bit of disease severity scoring that is rather a crude assessment. But with the patients who underwent splenectomy, and the patients who got diagnosed later with more disease burden, were almost more severe when you compare with the other patients who were diagnosed earlier, treated earlier, didn't have the significant disease load and actually splenectomized versus non splenectomized. So there are multiple feeders into the equation, how we are changing the phenotype and the degree of phenotype changed. But Dr. Ramaswami mentioned one thing, is very important here, is a point of no return. So obviously there is our minds tend to make us think linearly.

Meaning, if you look at a tissue or an organ, you are imagining the disease activity is all the same, all aboard among the cells, the tissues and with the different organs. This is not the case. Actually, the Fabry biopsies probably will be a testament to that. So basically, we see different disease activity, different disease stages within the same patient, within the same organ, within the same tissue. So, which means that the disease progresses as the substrate gets deposited, and it affects the newer cells or the cell tries to get rid of it. Some cells die and some cells will go into chronic effects. So basically when I counsel the patients, I emphasize this is especially important in patients with fibroid disease that comes with the adult presentation. So some of them will not be too warm to the treatment.

Obviously it's a middle age problem, meaning there is a family obligation, there is a job obligations, and there is something that is going to interfere or intervene with every two week infusions here. So we actually get all the lab results together. And then I have a patient with, let's hypothetically CKD, chronic kidney disease, stage two or two and a half. So when you start seeing the kidneys failing, initially more than 60% of the nephrons are probably already impacted. So you're looking into, as the time that creatine starts to increase, whatever is remaining left, you want to salvage the rest. And after that one point, there is more chronicity of the disease. And you deal with the complications where ancillary or secondary therapies become more important than the primary therapy itself, but you need to address it as the disease also, is progressing in some other tissues and organs.

But also Fabry disease is a great example to that, because when we compare the chronic patients with multiple complications, the advanced cardiomyopathy, kidney transplant, and all the later stages of the disease, these patients do not have increased substrate levels when you compare with the very young patient that is at the earlier stages of the disease.

Which means that the substrate deposition, the secondary effects has already impacted and did the damage. And now we need to actually mend whatever the broken car is left to us. So, that is my experience. I have another question about the timing or the start of therapy, and then actually what we are seeing is the phenotype difference. So could you comment on that too?

Uma Ramaswami:

So I think that's the million dollar question, isn't it? And I think this is where for some of the diseases at least, newborn screening, you very nicely showed in Pompe disease. For example, you showed that natural history, we've completely altered that natural history with the current disease modifying treatments. And that four year old boy was really doing remarkably well. And yes, of course, it's also a heterogeneous disorder, so not every patient is going to do well, but I certainly think for certain conditions, in my view, Pompe disease, MPS I, particularly if it's MPS I Hurler, you want to be transplanting them by the age of two. Yes, they have other complications. MPS I is a different phenotype with hematopoietic stem cell, which I didn't address today. I think that would almost be a whole talk, and the challenges we face as they get older.

But I do think that those children will do well, if there was newborn screening, for example. There are certain GD III phenotypes in my view will do well too. So there are few conditions where newborn screening will alter that. With Fabry, I think it's a little bit more challenging because of the number of variants of unknown significance and the later onset variants and how do we manage these patients? So I don't think one size fits all, but the sooner we start treatments, that's my view now Ozlem, having managed these patients for so many years now. My view is that the sooner we identify patients and treat them with disease modifying therapies, the better chance they've got in not having all of the substrate accumulation and the secondary pathways that we talked about being in the cascade that happens after that and triggering all sorts of other secondary problems. So, yeah.

Ozlem Goker-Alpan:

Yeah, absolutely. I agree with that. And then obviously the timing of the diagnosis and the onset of symptoms also matter in decision making. Obviously there are going to be physicians or the experts is going to be pro and actually against this, the reason is there is a whole variability in the patient presentation too. I guess, that leaves the expert that is much demand, meaning the patients need to be actually evaluated. And the decision when and how and what to treat also needs to be run by the experts in the area. The reason is, I think at the stage of my life, I can say, what is Gaucher, what is not Gaucher, and what is more important to focus on. One last question? Yeah, go ahead.

Uma Ramaswami:

No, I was just going to say the one thing that I do want for young trainees, perhaps who are on the call. I think newborn screening, the techniques are so easy. Now we can do newborn screening. We know we can have reliable cutoff for data on false negatives and false positives, and we can get these results. My main advice would be that we need to learn how to manage, not just the baby who's born with a condition, but also that family. And you diagnose them at birth. Now, these kids are going to be living, and this is a lifelong condition. Say for example, Fabry. We really need to have that infrastructure to manage that family, to support that family and also to not over medicalize. And I think it's a fine balance

and I think we need to address all this, but I do think there is a big role for newborn screening, as we move forward. With so many new therapies that are coming our way, I think that's what we need to be looking at.

Ozlem Goker-Alpan:

That is correct. I agree with that. So the newborn screening for lysosomal source disorders are the waters that is not thoroughly tested yet. And the universal, or even regional guidelines do not exist. So, we had a discussion about Gaucher disease yesterday and whether even it is feasible to do, or is it recommendable, to do newborn screening. Anyhow, in the context that we do newborn screening, and we find these patients with L44P homozygosity. So, these patients will have GD III, Gaucher disease type III. It is the prototype, GD III. And some patients actually were recommended to go and come back at certain intervals and let the patient start getting sick, which I am against this proposal. The reason is as we discussed, there is some point on non return.

So if the prognosis is already known, and so the sooner is the better. And I'm getting a comment here for Pompe disease, which is very correct. This is about the data, even between the start of therapy, between few days, make a whole difference. Like one week versus few days. But on the other hand with the newborn screening, obviously we are going to learn about what we can solve, which with these disorders and what we cannot, even with the gene therapy. Obviously some disorders like GD II may not be available to therapy because we have some evidence that our patients are affected already *in utero*. And there is a whole phenotypic spectrum in GD II. So I don't think there is any rescue for these patients. So there is a whole new area of discussion and treatment guidelines need to be established.

Uma Ramaswami:

Quite, I agree on that. And I also think on the later onset, even with Pompe disease, I'm not entirely sure whether newborn screening is going to address how we manage the later onset Pompe patients, and whether starting therapy very early will make a difference. I think it's so heterogeneous. So there are some ethical questions that need to be addressed amongst all this as well. Ozlem, there's a question here about combining ERT and I'm wondering, is it natural or I'm assuming it's new therapies.

Ozlem Goker-Alpan:

I guess we can answer this. I would like to answer as a combination approach. So what are your thoughts? Obviously as I showed in the picture, it is not one cause that causes the whole disease process.

Uma Ramaswami:

Indeed. And in fact, we have for Pompe disease, we do have the combination therapy, that's under clinical trials at the moment and going through regulatory processes. I do think adjunctive therapy. So that's the word I used, or combination therapies, I think will be considered in the future. For example, intrathecal with intravenous, all sorts of combinations. Those things are not clear at the moment. The cost might be prohibitive. The side effects might be prohibitive, but they are quite a lot of inflammation, but we do need to start targeting certain tissues. At the moment, the one size fits all, one treatment will

target every tissue, is not going to work. So I do think in the future, we do need to look at that kind of position, targeting therapeutics.

Ozlem Goker-Alpan:

So one last question here is the thoughts on the utilizations of multiomic approaches, like all sequencing proteomics, so and so forth for individualized therapy.

Uma Ramaswami:

No, Ozlem, you're already doing this, I know, in your center. And maybe you should address this, but absolutely. I think this is the individualized therapy. Even within families, I think we need to be looking at a multi-pronged approach in the absence of really good biomarkers, other than Gaucher disease. I really do not think at the moment we have good biomarkers. And so having that multi-pronged approach and maybe you can share some of the work you're already doing on this, Ozlem.

Ozlem Goker-Alpan:

So let's talk about the multiomic approach that we are looking into proteomics, epidomics, and the whole exome whole genome, the genomics. From this point on, actually, I want to have the minds change from a linear one or two dimensional therapy, or the therapeutic understanding of the disease, to a 3D approach. Meaning I think we can do proteomics, but what I find really valuable is input and output essays, meaning whatever we are doing, obviously we can't change the genomics except for the immunology. We initially actually decided I'm going to give you an example of the immune disorders or immune deficiency. So we decided to do a whole exome on every single immune deficiency patient that we have seen, or got treated with IBIG. And, unfortunately, yield was very low, meaning we got less than 10% hit even with the whole genomics. Which means that mostly is how the genes and the proteins, and then the cytokines interact with each other.

Maybe more meaningful than a steady state question and answer we are going to get. So obviously this is something really novel. I just read that there is a novel approach. You can actually say about 25,000 genes at a time, and you can find new, genes that will play in part in the diagnosis and the pathophysiology. So what are centers approaches to have a particular question and do the essays or design the experiments that is going to answer to that part of the question, because then what becomes is very generalized. And I get what I'm asking for. I'm going to give an example of MPS. So these disorders are inflammatory disorders. So many people are asking what is the role of inflammation. And then we are doing proteomics and trying to find the responses, but IL six is sticking out.

So IL six is the very end of the pathway. And it's almost that universal response. So having an IL six increase and having IL six therapy employed probably will not be the cure or even treatment for these patients. COVID gave us an example for that. IL six, obviously they were coming akin to cytokines storm. So why don't we give IL six? So the therapeutic response was very poor in these patient population, and didn't really changed the phenotype or even outcome. So basically, I guess we need to understand the 3D model of the disease or the interaction of these proteins. Obviously we are very early stages for that, and we need some young and very sharp minds to further this.

Uma Ramaswami:

I agree completely. And I think the way we manage patients is going to change quite significantly with the multiomic approach. But I think that's not just the answer because it's going to give us so many questions that we are not going to be able to.

Ozlem Goker-Alpan:

Well, that is why there are multiple things – AI – there are multiple techniques are coming and the real big word data, the issues that are related to the disease. So basically, and one very important aspect., we are clinicians here that also do some lab work. What is needed in this area? What we call is the translational scientist, meaning that understands the cellular environment pathology and can understand, interpret what a lab result is, in addition to the PhDs that on the diagnosis. The reason is if the right hand doesn't talk to the left hand, then Fabry disease is a great example to that. When I go to these meetings, we are still looking into the echocardiogram, the MRI, so and so forth, but obviously that is very important, but then we need to address how we can improve it, what we need to do. And what is the course and what is the basic science to this? I think that is very important. And for the future, we need the physicians and the physician scientists that work together to understand the disease pathology to develop novel therapies.

Uma Ramaswami:

Yeah, I agree. And I think there's only just one last question. If I may. I'm just going to be very brief on this and just say, this is about whether a child who survives after two years, we now switch from calling the child the condition as GD two, and it becomes GD three. I'd like us to move away from GD two and GD three, and talk about neuropathic and non neuropathic negotiated disease. In fact, non neuropathic, even that has Parkinson's, but at least a clear distinction in childhood. And then there is a continuum and there is a severity spectrum within the neuron apathic Gaucher disease. And I think that would be my view.

Ozlem Goker-Alpan:

It's agreed. There is a whole spectrum rather than a single, straight definition of it can apply to any disorder. So with this, I would like to take the opportunity to think Uma again. Let me just quickly go for through the questions, whether we did not answer any major one, but I think we may have covered all of them. Yes. Is the child, what do we change it? A child expanded lifespan. All right. Thank you Uma. That was a wonderful presentation.