Advances in Gene Therapy for Lysosomal Disorders.

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Hi, everyone. On today's CME series on lysosomal disorders, we are going to be discussing the advances in gene therapy for lysosomal diseases. These are my disclosures. Today, we will describe the limitations and unmet needs of the current therapeutic landscape for lysosomal storage disorders, review the principles of gene transfer therapies for lysosomal disorders, describe the best clinical practices to monitor the safety profile of the gene therapy, and describe the best practices for gene therapies in general.

Anyone that has seen and treated a patient with a lysosomal disorder should be aware how the treatment and the treatment response get challenging for a given patient. The reason is, it is not one pathology contributes to the whole clinical picture, but multiple pathophysiologies actually end up what we see as the clinical spectrum. From the disease-causing mutation, we have a disease-associated protein that can go either way; it either gain of function, and also loss of function at the same time. At the end, that's all the disorders actually converge into the inflammatory response with immune dysregulation and inflammation.

The current therapeutic landscape for the LSDs include obviously enzyme replacement therapy – that has a past of more than 25 years – that started with Gaucher disease. In general, the exogenous recombinant enzymes have mannose 6-phosphate tags that are recognized by the mannose receptors and taken first into the tissue and then shuttled or trafficked to the cell, they overcome the catabolic pathway, block and affect the clearance of the store substrate. But as we all know that the enzyme replacement therapies are all large protein, large biologics, and mostly, they do not cross the blood-brain barrier.

This actually takes us to the small molecules. Small molecules, obviously, they're chemicals. They can disperse in the multiple tissues and in the bloodstream. Given time, they actually have a steady state, so they can overcome the peaks and valleys that we see in an enzyme replacement therapy. They, in principle, reduce the production of a substrate by inhibiting the upstream pathways. Some small molecules, they may cross the blood-brain barrier because they're small enough, and they use the appropriate channels and receptors. These small molecules... actually in general, we are going to be talking about substrate reduction... but also they include chaperone therapy and some other small molecules such as cyclodextrins, cysteamine, so on and so forth.

In general, when we look at the current landscape of LSD treatment, obviously these are the disorders we are going to be reviewing... MPSs, Pompe disease, Gaucher, Fabry, and Krabbe. We have the FDA-approved enzyme replacement therapies that actually exist for all these four disorders that we discussed. In human trials, there is this CNS administration of ERT, or brain-penetrant ERTs, that's been approved in Japan for MPS II. For Pompe disease, there's the new ERT that got recently approved in U.S. last year, in August to be exact.

Obviously, we are also still conducting trials using enzyme replacement therapy with a chaperone. Also, the human trials are underway for gene therapies that include for Pompe

disease, upcoming therapies for Gaucher disease, and obviously Fabry disease gene therapies have been happening for a while.

In clinical stages, all these disorders, they also have preclinical development of gene therapy and also hematopoietic STEM cells have been used as off-label for Krabbe disease and other disorders.

In principle, the enzyme replacement therapy, they may treat systemic manifestations of LSD. There is one intrathecal form of ERT has been approved for Batten disease, actually, the intraventricular administration, that bypasses the blood-brain barrier.

But obviously, there are limitations of enzyme replacement therapy. We see hypersensitivity reactions that may range to a simple allergic reaction to full blown anaphylaxis. Obviously, the ERT is very short-lived in the periphery, requiring repeat dosing. And also for neuropathic forms, you need to administer to the brain, which is very invasive, and efficacy is affected by limited tissue penetration that include lack or very limited tissue penetration to the lungs, to the bones, to the cartilage, so on and so forth.

When we talk about substrate reduction therapy as a small molecule, these are in general what we are talking about glucose ceramide synthase inhibitors or glucose ceramide synthase pathway inhibitors. There are approved therapies... a substrate reduction therapy for Gaucher disease... but also there are trials that are underway for treatment of Fabry disease, Tay-Sachs, Niemann-Pick, and GM1-gangliosidosis. For Fabry disease, there is also chaperone therapy recently approved that is included in the small molecule therapies.

Let's switch to gene therapy. From this point on, I would like to briefly talk about the mechanisms of gene transfer therapies. We are going to be actually focusing on the AAV-based gene therapy or gene transfer therapies. I'm going to briefly mention about the ex-vivo gene therapy, but what I want to focus from this point on is mainly the immune response to the gene transfer therapies, and also the mitigation of this response to prevent the complications. The reason is both the efficacy of a gene therapy actually depend on immune response, but also any clinician that will be following patients that have been administered gene therapy should be aware some of the complications related to gene therapy and need to learn the timelines for this complications, potential complications, and also how to mitigate that.

When we talk about gene therapy today, we are basically talking about gene transfer therapies. In principle, there is the in-vivo, where the gene product is transferred into cells while in the patient, and the other technology is ex vivo, where the cells are taken from the patient, a certain cell type, the genes are modified in the cell, and then the cells are then transferred back to the patient.

When we look at obviously the genes are in a very simple saying, they're transferred using the capsid of a virus that has the potential to infect the multiple tissues, but obviously they are rendered "not harmful". When we look at the vectors in general, this adenovirus-based vectors are the primary means of introducing genes into the humans right now. When we are looking at these trials, AAV-type viruses actually are the primary means being used as vectors. This is a 2019 reference and there were more than 244 trials. When I look at different kind of resources, the current trials range between 159, with more than 50 having a preliminary outcome measure.

Very briefly on hematopoietic stem cell gene therapy, or ex vivo gene therapy, this actually had been approved for monogenic blood disorders such as SCD and beta-thalassemia. Gene transfer occurs in autologous hematopoietic stem cell progenitors to treat the monogenic disorders. These are CD34+ positive T-cells and gene-corrected HSPCs, and their progeny can be also excluded as gene cell vehicles to deliver the molecules into the circulation and tissue, they also have glial cell penetration, and gene editing technologies such as CRISPR could be exploited in this kind of gene therapy.

Let's switch the gears and actually dedicate the rest of the talk on AAV-based gene transfer therapies. Why AAV is so broadly used right now? Obviously, if anybody who should remember the timelines of gene therapy – when the retroviruses were used originally – actually, that's the catastrophic first trial for a urea cycle – halted the gene therapy because of the fatality associated with it. For some time we did not have any meaningful result for a gene therapy protocol. However, from the beginning of 2010s, the animal studies coming about the safety of AAVs..

AAVs is short for adeno-associated viruses – basically, these are faulty viruses and they made pathogenicity. But they are very efficient in transduction into non-dividing cell. Also, the AAV genome can persist for a long time after a single administration in the animal studies.

There are multiple AAV strains with different tissue affinity, and in the approved treatments AAV2 and 8 have been used for ocular therapies, and also AAV vectors are versatile and can be designed to target various tissues, by the tissue affinity of the particular serotype. Among these, AAV2 and AAV9 are the muscle and CNS targeting, and AAV9 can cross the blood-brain barrier. AAV also can be designer-made, so they can be custom-made by mixing and matching vector elements to target specific tissue types. These are also called designer AAVs.

However, all these properties that makes the AAV very amenable for a gene transfer therapy, can also be its rate-limiting factors. They have limited cargo capacity and also there is a widespread pre-existing immunity to the AAV. It is a small genome. It's 4.7 kB with an inverted terminal or ITR sequences and two genes in Cap and Rep. In very simplistic terms again, to manufacture AAV vectors, this Cap and Rep are removed and then Cap, Rep and helper proteins are expressed from other DNA sequences. Basically, you express the genome of interests so the virus can transduce, but cannot infect in the terms of basic viral pathology.

From this point on actually we want to talk about the immune mechanisms and vector attributes. The reason is, we discussed or we said that there is a preexisting quite widespread immunity against AAV vectors. From the previous studies, we know that the immune-mediated toxicities have occurred in several trials that had been attributed to empty capsids or hypomethylated CpG motifs. Similarly, AAV vectors with different design elements, aka the designer AAVs, may lead to differences in product attributes, and may play a role in triggering the immune system and the immune reactions that is from immediate hypersensitivity to complement activation and antibody, formation have occurred.

Pre-existing humoral immunity to the vector is about 30-60%. In our experience. – we run a few gene therapy trials currently – it's about 50-57%. The seroprevalence of the neutralizing antibodies against the wild-type AAV varies also geographically, and also for some disorders, I think it's more prevalent than the others. Titers of IgG type antibodies correlate with the

neutralizing antibody titers. Also there is a broad cross-reactivity among the AAV species, so if a trial is run by a certain type of AAV and also the patient has antibodies against that AAV, there is a likelihood the patient will have antibodies to other AAV species too.

Antibodies can be actually mediators of toxicity – that is the complement activation. Currently, there are no trials that actually utilize, or that is done, in patients with low titer antibodies.

There is also pre-existing T-cell immunity that is less studied than the antibodies. Exposure of PBMCs to the AAV capsid epitopes induced CD8+ T-cells with effective memory, excreting TNF alpha, granzymes, CD107a, and also there is transient activation of TK cells in seronegative patients.

If a patient doesn't have existing immunity, what happens when we infuse the gene product? There is an induction of human response to the vectors that is towards the capsid, towards the genome, and towards the transgene products. All these three different elements can induce the activation of NF-κB and interferon regulatory factor with the release of type I interferons, that in turn induces the T-cell or adaptive immunity. You start with the humoral response, but you end up within a universal T-cell or adaptive immune response. Basically, there's a cytotoxic CD8+ T-cells drive the clearance of AAV-transduced cells. Basically, when we start losing the AAV, then it is the humoral response switching to the T-cell response.

We said also there is the transgene product – the response that can be towards the transgene – that actually interplays between the host factors, which is the pre-existing immunity, that is the disease state, which is the inflammatory state. As we discussed, all the LSDs are inflammatory disorders. The genetic background also among the host factors, and also the vector factors such as the serotype, target tissue, vector dose, purity, so and so forth, and also which organ that we are targeting. The combination of the AAV capsid, the vector delivery route, and whether it's local, such as to the eye, or you give systemic administration, obviously there is more immunogenicity.

These are some of the examples of the immune responses. Obviously, that's just a look at some of the safety of previous AAV gene transfer therapies. The gene therapy for SMA have been actually approved. What they have in the trials, there are patients with elevated liver enzymes, but there are reports recently from Eastern European countries that there are actually two infants have died of acute liver failures after the gene transfer therapy for SMA.

When we look at the other safety issues or the SAEs, they include reduced platelets and RBC complement activation, acute kidney injury. In the recent DMD trials. – actually, one of the Duchenne muscular dystrophy trial – had been halted the past winter because of all these reduced platelets or RBCs. They're also called TMA or thrombotic microangiopathy – that can range from aHUS, or atypical hemolytic uremic syndrome, to one of the LMS, either reduced platelets, or you can see actually reticulocytosis because of the destruction of the red blood cells – also kidney involvement with the capillaries with proteinuria, so on and so forth.

When we look at the safety of the AAV gene transfer therapy and the timelines that include the pre-existing immunity... so this actually precludes the administration of gene therapy. If the patient doesn't have the pre-existing immunity, and then the gene therapy product is administered to the patient, in the immediate, obviously, there may be acute allergic or immune hypersensitivity reactions. But when the innate immunity gets activated, acute

toxicities that can start between 24-48 hours, up to four days, and can last up to 15 days. What we are looking here is the activation of the complement system and the impact of the complement system activation to the end organs that include kidney, lungs, heart, so on and so forth.

From four to eight weeks, the adaptive immunity from the innate immunity takes over, as we discussed. The cytotoxic T-cell responses, basically with the pro-inflammatory state, actually decreases the efficacy of the transduction of the gene therapy product, that can lead to high-titer neutralizing antibody formation and then the transgene immune responses.

The potential solutions to the innate immunity activation, basically inhibition of the complement and IgG absorption, and for the long-term for the transduction efficacy, we are talking about immune suppression, as a general strategy that is going to suppress the innate and T-cell mediated immunity.

From this point on, I would like to invite Dr. Sonata Jodele who is research professor at Cincinnati Medical Center. Dr. Jodele's research had been focusing on the immune activation, the complement activation, that leads to thrombotic microangiopathy. She's an internationally recognized expert in transplant associated TMA and her groundbreaking research is rapidly transforming the way we perform the gene therapy.

Briefly, she received her medical degree from Vilnius University School of Medicine in Lithuania and got her board certification in pediatrics in SUNY Health Sciences Center at Brooklyn in New York, and in pediatric oncology at Keck School of Medicine. She is, as I said, currently working as a research professor at Cincinnati Children's Hospital. I'm going to give the word to Dr. Jodele to talk about TMA associated with gene therapies and the mitigation strategies. Thank you.

Sonata Jodele, MD

Thank you very much for this nice introduction, and thank you for inviting me to your meeting so we can learn from each other's disciplines. I will discuss thrombotic microangiopathy as one of potential complications in gene therapies.

Here are my disclosures.

Thrombotic microangiopathy is now quite well-recognized potential complication of gene therapies, especially adenovirus-associated gene therapies. DCMS can present with variable severity and been observed in multiple studies already, like for patients with spinal muscle atrophy, Duchenne muscular dystrophy, Diamond disease, including a case of very fatal TMA in a young child who had likely underlying complement defects.

TMAs as a complication has been addressed by FDA and European agencies, so we're striving to learn more so we can prevent those events from happening. To our understanding currently in gene therapies, TMA can occur about within one week of initiation of therapy. Usually, the earliest marker is being thrombocytopenia, elevated LDH, and terminal complement activation that could be measured by elevated soluble C5b-9 in the blood. TMA usually aborts within a couple weeks, but it poses high risk for organ injury due to severe complement activation. Severe complement activation has been implicated as potential culprit of TMA-associated organ

injury in gene therapies. Those organ injuries present in a kind of similar pattern to what we've seen to other TMAs, and I will share some experiences with you today.

Gene therapies are promising therapeutic options, and we want to keep our patients safe. We want to identify those preventable complications. With TMA, we could do that with rigorous screening for TMA, and also we have complement blocking agents in selected cases that need it. Ultimately, we would like to double up screening and prophylactic strategies so we don't see this problem. Definitely, we can learn from each other.

For those who are less familiar, any type of TMA belongs to a big group of disorders under complement-mediated disease syndromes – glomerular, aHUS, TTP, transplant TMA, HELPP and eclampsia – all those disorders fall under same umbrella. They usually present with microangiopathic hemolytic anemia, but more importantly they all have final common pathway of endothelial injury that presents as certain organ injury that could be very severe.

To remember the complex complement cascade that we all studied in college, medical school, and we know that there's classical lectin and alternative pathways that they all lead to terminal complement activation. This schema shows a little bit of the drugs that we're learning about right now – mostly C5 blockers, MASP-2 inhibitors, C3 blockers.

Why I'm bringing this picture up? Complement system overall is a good thing because it's part of our innate immune defense. But it's almost like a fire. If it's nicely content and doing its job in the fire pit or fireplace, it's good. But if it gets out, then it's overwhelming. It usually has very severe complications. So we want to know how to identify and how to control those surges of complement activation.

Here's a big list I will not go over, but as you see, complement inhibition is a big attention of research right now. Multiple disorders have complement system involved. The idea is to identify when you need to intervene, when you don't, and when it's beneficial. We're learning, like I said, from each other.

Some novel thoughts on the complement activation and inhibition is coming from different disciplines. For example, we're seeing severe complement activation in the hypoxemic brain death. This is really significant for organ donation, outside of other clinical situations where you see significant hypoxemic brain injury. We see it in the vascular emergencies like MI and stroke, when you have a complement surge and you have complement deposits in the cardiac tissue. Those are kind of new fields that we're learning more, and those are very acute emergent field where we don't have too much time to think. We need to know what to do to these patients. We also are seeing a significant complement activation in traumatic brain injury and also demyelinated disorders. It has been kind of clearly showed that complement can be deposited on injured neurons. Again, this is another target we could think about in certain disorders or certain emergencies.

We're learning a lot about complement from our current pandemic with SARS and COVID-2 infection, as it's been shown that complement system is really heavily involved – especially lectin alternative pathways... is heavily involved in the patients with COVID infection and who present with thrombotic microangiopathies. As you see here in the figure on the right, you see very significant complement deposits in the vasculature and endothelium in multiple tissues of COVID-affected patients.

Today, I'll concentrate a little bit on the field that I am doing research and I'm most familiar. It's a transplant-associated thrombotic microangiopathy. Namely, I'm talking about stem cell transplantation, not solid organ transplantation.

In our patient population, TMA could be a very significant, severe and life-threatening complication. Very often, outside of presenting this hemolytic microangiopathic anemia, it really presents as multi-organ injury. It's kind of classic presentation. Although we have such a complex patient population that we have to work with multiple diagnoses, but we could share some of the learning points from our experience.

TMA incidence is pretty high in our transplant population. In allogeneic stem cell transplant where a donor is other person, usually up to 30% incidence of TMA occurs in children and about 20% in adults, to current reports. In auto transplant, where donor is itself, it's less known, but we know certain diagnosis like neuroblastoma in pediatrics get their high incidents of TMA.TMA quite often goes with high-risk features which would be complement activation and organ injury, and about half of any TMA presentations could be quite severe. If you have a severe presentation, this organ injury mortality is very high – it could be over 90% mortality – so it's a very challenging disease to deal with is their very high mortality.

Today, gold standard for TMA is a tissue diagnosis, usually kidney. But as you see here on the top panel, kidney biopsy is quite classic. In any TMA, it would be looking quite similar, where you have thickened capillary walls, fragmented red cells stuck in a vascular lumen, endothelial injury on electromicroscopy. In a systemic illness, you can see that in any tissue, like a long bowel drain. The challenge is that biopsies are quite difficult to get in the patients who are very acutely sick with thrombocytopenia, other organ injury, or if TMA is occurring so acutely that your patient is getting so sick and you don't have that opportunity. We, so far, rely on a clinical diagnosis using laboratory tests. We're learning to recognize clinical organ injury patterns.

From a laboratory diagnosis, TMA, in the transplant population, is defined by more than four of those seven listed criteria, that includes elevated LDH above normal, schistocytes on peripheral smear, de novo thrombocytopenia, anemia or requirement of transfusions, arterial hypertension that is excessive for age and therapy that is given, proteinuria, and complement activation measured by soluble C5b-9. Proteinuria or complement activation had been shown multiple times to be a high-risk disease feature.

Classic organ injury, you need to think among differential diagnosis. TMA is a vascular endothelial injury. It will be a primary endothelial injury usually presenting as oxygen exchange in the lungs, let's say, or as bleeding in some other organs. In intestines, it could be ischemic colitis with pain and bleeding. In lung, it could be hypoxemia, pulmonary hypertension. In CNS, again bleeding, seizures, PRES. It could be even in the skin as vasculitis. Technically, it can be in any organ.

As I mentioned, histologic tissue evaluation would be great, but it's hard to get, so it's not really mandatory. If you have it available, it's good. Complement screens could be also done in any tissue. But I wanted to mention that for TMA who evolve very fast, and have fast progression, and you sample tissues right away, you might not see complement deposits because it takes time to deposit complement. If someone presented acutely, you got tissue, you screen for

complement, it might be negative. That does not exclude TMA because it has other histological features that you can diagnose.

Now I would like to think about parallel between transplant TMA and HUS. In aHUS, atypical hemolytic uremic syndrome was kind of leading disease in complement drug applications, and they usually propose lifelong therapy, and we treat our patients for short time. The differences are that we think that patients with HUS have a complement gene mutation that predispose them to vulnerability under any stress to develop this disease.

In our transplant population, we show that patients have usually complement gene polymorphisms, and under a regular life situation, usually they're not very significant. But if you subject patient to a very high stress, then those TMA can occur. Usually, when stress is over, TMA resolves. We don't have a chance to double that again, and we're able to treat those patients for short time. That's what I think would be also applicable to other therapies, that you have expected high surge of cytokines or stressors like gene therapies probably.

We wanted to see, can we improve outcomes in our patients? We knew that the high-risk patients have really poor outcomes. We looked at all TMAs. We screened them prospectively in our institution. Low-risk TMA was those who had just hematologic TMA without proteinuria or without complement activation, and got no targeted therapy. The high risk had both features; activated complement, proteinuria, and usually multi-organ dysfunction, and moderate with dosing in the middle. We use urine protein-creatine ratio of 2 mg/mg as a high-risk feature because that is a level of nephrotic proteinuria which is very high.

We also discover through many studies, prospective and retrospective, that complement activation, namely terminal complement activation, measured as elevated C5b-9, is a high-risk feature of TMA, as I mentioned a couple of times. We show that using complement blockers – namely in pediatrics we use C5 blocker eculizumab, we have most experience – and adults use MASP-2 inhibitors, which is lectin pathway inhibitor – we can improve outcomes in those diseases. We also showed that the higher C5b-9 activation at the start of diagnosis, the more challenging to treat the disease, and it takes more medications to get the disease under control. Procrastination, usually, in those cases is not in favor of outcomes.

We performed a study on those patients also. We looked at the patients who did not get complement blocking drugs, but they had moderate risk features. We really determined that if we screen for TMA, we can really identify those patients in advance. We again supported our prior observations that elevated C5b-9 is associated with threefold higher risk of mortality. We also identified that each patient has a baseline of what the complement activation is. It's not really a laboratory value that matters, but where you escalate from your baseline. We show that our patients with double C5b-9 number from their pre-transplant baseline, and if they sustain it for longer than a couple of weeks, they usually have a risk of high-risk TMA, this multi-organ injury. We also showed that the long-term outcomes like renal outcomes are better even for those survivors, of course, who were high risk TMAs but treated, as compared to those who had a moderate TMA and were not treated. Definitely we can make a difference here for long-term outcomes.

The most experience we have is the drug eculizumab, which is not approved indication for transplant-associated TMA. It's approved for aHUS, PNH, now myasthenia gravis, neuromyelitis

optica spectrum disorders. It's a humanized chimeric anti-C5 monoclonal antibody, but we adopted its use in transplant-associated TMA with some adjustments.

Here is a busy figure, but I just wanted to show you. If you look first at the red line, that were our outcomes so far, for our patients, before we ever treated them for high-risk disease. I'm going to mention again... only high-risk disease patients were offered complement blockers in our field. The moderate and low risk were not treated, so low risk as you see, there aren't interventions... they're doing well. Moderate, they have so-so outcomes. And the high-risk disease... now these therapies we moved them up to about 66% survival from 16.7% survival. Overall survival post-transplant was 9% in those untreated patients before, so it's a good improvement, but we're not there yet because complement again, is not the only one pathway involved in TMA, and we still need to learn how to use it in less severe disease. We're doing steps right now to improve those outcomes further.

Here's a quite busy schema but I'm just going to deliver thee main message. First, if you look at the left figure, it just demonstrates that C5b-9 is a marker that is associated how fast your drug, like eculizumab, will be cleared out of the system. MABs are usually target-based medicines and they depend on how many targets are there, so more targets, faster drug clearance. We use C5b-9 marker for TMA diagnosis, as I mentioned. We use this as a risk stratification or prognostic feature, and we also use it to assess therapy outcomes because as it normalizes, it shows that C5 generation from affected tissues is subsiding.

The other very useful marker is CH50, which is the figure on the right. It's total complement activity. First of all, when we didn't have eculizumab drug levels available, we used it as correlative measure for complement suppression in blood. If you give a drug and CH50 is suppressed below 10% of normal value, that means your complement is blocked in the blood. It's a very good feature and a very good lab to follow if you stop the therapy. You want to do antimicrobial prophylaxis until CH50 normalizes after therapy stopping because that indicates that complement activity resolved and reestablished. Those are very good markers to use.

Here's a very quick illustration again on a busy figure. Eculizumab clearance also depends if patients have intestinal bleeding or not. We, in our field, have patients with intestinal TMA who have very significant clinical bleeding for days and days. Patients without bleeding usually, as disease control happens, we slow down drug clearance, and then they can stop therapy. Patients with bleeding need much longer and much more personalized therapy. We're developing different algorithms and calculators how to calculate the clearance of the drug based on patient's clinical condition. Just an example of this calculator-shown clearance curves on the right side.

Again, I just wanted to mention the concept per se that eculizumab and other complement blockers could be mABs and they depend on the targets how they work. If you have a target, they will be consumed and used, and if you don't have a target anymore, they would not be used as a drug because there's nothing to block. That's why when we have active TMAs like the spore is burning, we need lots of drug and intensified regimens, and we use intensified regimens – to compare to HUS – because our transplant TMAs are much more active. But when process is over, there are no targets anymore, we stop the therapy because there's nothing to block anymore. It is good that we have those tools to tell.

Now, I wanted to mention about complementing T-cells, because we use so many therapies where we have immunomodulation in transplant and other therapies like gene therapies.

Can we use complement blockers safely? We show that we can use it safely because T-cells usually have what they call complosome internal T-cell complement system that works, and a C5 blocker like eculizumab does not block it, so their T-cells are functioning. It was very important to know. T-cells also work with an interferon system that talks to complement too. We're learning those things; how to use those comprehensive complex therapies together so they don't cancel each other.

We also showed that we can use other T-cell therapies like antiviral T-cell, It's a busy slide here too. But the point is here that we used complement blockade, this antivirus specific T-cell therapy, because those patients often have TMAs. We demonstrated that under complete complement blockade, those T-cells extend, and they do the work that they're supposed to do, as it would be in patients who are not under complement blockade. Those are very important observations because we want to make sure that we are not using therapies that cancel each other.

What could be a suggestion for gene therapy TMAs for management? I think one of the suggestions that I have and I strongly feel about, it should be a TMA screening. It's very easy to do, we have those labs... majority of them at least. It's a CBC with differential, looking for platelets, and hemoglobin, and schistocytes, and LDH, haptoglobin. A random protein-creatine ratio is a very, very good marker of a kidney injury in a TMA. Hypertension is a very good marker proven many times. And also terminal complement activation. The slide is limited to some major institutions, but it could be done on pretty fast turnaround.

Now, if one has impending TMA, I think in gene therapies, if someone has new thrombocytopenia, complement activation, that's a big signal. And then if you have another microangiopathy marker, or associated organ injury, those patients should be assessed more for TMA and considered for therapies.

What can we do better now? We can together improve awareness. We can work on early diagnosis, this prospective screening, and recognizing organ injury. It's very important to educate your multidisciplinary collaborators like kidney doctors, nephrologists, cardiologists because you need an ICU team to be involved and know about those patients, and learn can we apply early interventions in certain selected patients who would benefit and definitely long term survivors because effect on the organ is for a long time.

Here's just to conclude that complement is not the only pathway. We know that other pathways are involved in the TMAs right now, and as we gain more medications, like we're adding our knowledge, hopefully we'll have a comprehensive multidrug therapies for those patients in the future. We know that interferon pathway is very important and we have interferon gamma blockers right now. We know that neutrophil extracellular traps are very important, especially for lung injury. Coagulation system is very important. Certain viruses like BK viruses and other viruses can trigger. We're learning and we're adding to this knowledge, so it's not going to be the only pathway. We learned most about complement because therapeutics were available. We took that opportunity to learn and to apply and make a difference.

There is also interactive way to learn about TME. We created this very acute cartoon for our young patients to explain complex problem like TMA. I invite you to watch it.

With that, I would like to say thank you again for inviting me to present, and I would be happy to answer any questions. Thank you very much.

Dr. Goker-Alpan

This was a very actually informative session and I am sure we are going to have many questions. I have few here so I'm going to start with few of those, and then if the time permits, I will add few more.

First, the question is from one of the attendees.. It says "what about the rest of complement factors? They can mediate inflammation without the terminal complement activation, and can contribute to the TMA or any other complication associated with gene therapies?"

Would you like me to answer that question, I assume?

Dr. Jodele

Thank you very much. So yes, we are learning about full casket in different parts of the casket. So idea would be in the future to target only what's needed, if we can identify specific pathway of complement pathway target that could affect particular problem and solve it. There are some disorders that we know already that blocking at C3 or lectin pathway C1q could be beneficial. But we started learning, kind of from a bottom, because that's where endothelial injury is, at C5-9 level, that's where physical attack happens with MAC complex. So we blocked at this and some parts of the complements still remain unblocked higher, which probably gives reasonable exposure, and fight for infections. We don't get fungal infections usually with C5 blockade.

But in the future I think we're going to learn probably for particular disorders, for particular conditions, that maybe we can be more selective and we're striving to do that. But so far, I think best will we have in this it's terminal blockade so far, or lectin,like I said, in adults, if we have lectin exposure. But we have the best handle on C5 blockers.

Dr. Goker-Alpan

Okay, so I'm going to ask you actually a practical question. So we had few patients who had laboratory evidence of TMA of varying degrees. Unfortunately, the complement levels take a long time to come, and whenever you need to make a decision. So what is the best indicator of microangiopathy among all the parameters that you discussed?

Dr. Jodele

In our world, as I was presenting, any complication, we have a different level of TMAs. So in the transplant population, the stem cell transplant population, we showed it quite well that if one has only hematologic TMA without complement activation, usually these patients resolve TMA

without any interventions. So in our hands, complement activation is a very good feature to tell who's a very high risk and will get in trouble with organ injury. Proteinuria, again it could be just a lung TMA or other organ TMA; not always kidney should be involved. Complement activation is one of the very significant features for risk. I understand that in your patient population, things evolve so fast and you have a brief period of time.

Technically, it's very likely to be a complement-activated disorder. So if you see features already like significant thrombocytopenia. LDH, or if patients getting real sick, you almost can assume that complement be activated. I would send a lab, and probably if patient's really ill and you can start therapy, you can give a dose of a complement blocker, assuming that all conditions align and physicians decide on that. Everything is presented on assumption that you need it. And then you can get your labs back in that time. Those labs are getting more available and hopefully will be more available in time. But so far complement activation is the best marker. You don't need even to align all TMA markers. If you have let's say significant thrombocytopenia, let's see some LDH, or some kidney injury and complement activation, you definitely should think that this patient at high risk for injury.

So we hopefully will evolve some panels but we're working on that to determine. In transplant population, hematologic features are not as important probably as complement activation and organ injury.

Dr. Goker-Alpan

So actually in those patients, we proved that there was complement activation but obviously the complement levels came a week later. I'm going to talk to you about a specific case that actually I think we had discussed. So basically, there was progressive proteinuria in the patient with evidence of increased LDH, reticulocytosis, and relative thrombocytopenia. I mean that was never in a dangerous manner, but we had increasing protein levels after the nephrotic levels. In such cases, we know that the protein is increasing every day so there is some likelihood that there is kidney capillary involvement. So when is the time to consider... because you're going to do yay and nays... is that right for the eculizumab therapy... then probably to stop the cascade of events. So when do you need to start considering, and what is the timeframe?

Dr. Jodele

Million dollar question. But I would say if you see only lab values that are not very significant changing... thrombocytopenia should be measured probably in a percent which you're dropping your delta. If you are going from platelet count of over 400,000 to go to 150 is abnormal. it's a pretty big drop. So we usually kind of offer to think if one drops more than 25% from what the baseline is, it's a significant drop. Seeing this hemoglobin, if you drop by a gram of hemoglobin – since your patients hopefully don't have other underlying conditions at that point that would affect let's say platelet count, hemoglobin, transplant that we're seeing is mixed. But in my world, if I would be seeing impending organ injury of any kind, I personally would be worried knowing what I know.

Again, these strategies have to be agreed between the groups and approved. It's a bit easier for me to say because we deal with very acute lethal disorder in bone marrow transplant population and when we already see impending organ injury, or evolving organ injury, you know those things will not really abort itself unless you intervene.

If one would take a complement-blocking agent only as an agent, taking away availability and cost and all those other issues, it's a reasonably low-risk drug if you prophylaxis for infections of course and make sure that you take steps not to get meningococcal infection. But usually if you block it, complement for whatever time needed and then later blockade is resolved, you do not have permanent damage to innate immune system. It might not be a best comparison, but when people use steroids in medical field, yes it's also immunosuppressant, it also affects lots of things, but if you use for brief time where it's needed and you don't use it any longer, then you are not really experiencing lots of side effects and you alter or control immune system just for brief time.

But I would say as a physician I would be worried if you are seeing any clinical condition going towards organ injury. I would be worried. Especially those stereo classic organ injuries that that's where we're trying to define them; how they look, how to recognize TMA-associated organ injury,because other complications can occur, yes. If someone is needing oxygen and oxygen requirements are increasing and lungs look semi-clear, not like a fluid overload, it's likely it's a vascular injury.

So you have to be a good clinician to differentiate those on the spot. The clinical tools and clinical assessments are very, very helpful if you understand primary pathogens of TMA and how organ injuries should present. So just training yourself to recognize those.

Dr. Goker-Alpan

Thank you. So there is one question about whether there are any less costly alternatives. I will actually tweak that question a bit. Obviously, when there is organ injury, and whether we can prevent it, maybe cause is not the most important thing that we need to think about. But are there any mitigation strategies for the milder cases that can potentially prevent the end organ damage?

Dr. Jodele

Short answer of complement blockade and mediation, probably nothing that directly affects the complement blocker. If you have a pathway activated and you need to control it, the best way is controlled with exactly targeted therapy.

Now, we're working understanding other pathways because complement does not work in isolation. This works with interferon system and neutrophil extracellular traps... different systems. Nothing so far that you could physically giving one medicine, that is not complement blocker, to block the complement, that we could see it. We paid attention to some steroids... because steroids work through the interferon system and that would be a mediator. Unless you do it very, very early, maybe could affect a little bit, but not as impressive as the complement blockade. So I can't speak to steroids that they would be a substitute.

We looked at prophylactic measures, even like N-acetylcysteine, like NAC, to prevent TMAs. Some vitamins even modified... like vitamin D modified, vitamin A, vitamin C modified endothelial health... but we are talking if you are evolving TMA probably as a more slow process, you could probably prevent these drugs. If you have a very severe surge, like you see in the gene therapies... very acute and very severe... it's probably not very likely that at this moment is what we know that something else would control complement as fast, and as proper as a complement blocking agent. Not saying that other immune modulators could not, but if you really need to be very fast and very precise, that's the only way, so far, to go.

But hopefully we'll get that answer in the future. If we can get the combination of medications, or I think that preventing that from happening would be another really important area that we should be working. Maybe we can modulate something upfront. How these patients come into the procedures, what immunosuppressants you use, all those modulations could affect your vectors, all those modulations could affect what TMA presentation you will have. And if you have just a little bit, it's not going to hurt you but if you have a very severe, it will hurt organs. So I think it's working process, we'll get those answers.

Dr. Goker-Alpan

Okay, thank you. I think the time is up. I just have one more question. So one of the attendees is asking how can we tell early on if the case is going to be milder? So we just procrastinate as opposed to we start getting the eculizumab ready. So, is there anything to differentiate? Obviously the complement levels is one, but any other clinical indicators?

Dr. Jodele

Even if you screen it with all hematologic markers, never say no. Like we're discussing at the lecture, if you know your patient's baseline, how you're coming into the procedure, and just the change from your baseline, tells you really a lot. And if you look at the one time point for TMAs, if anybody gave you one time point of labs, it would be hard to say what happened. But if you have a pre-procedure baseline, and then you see how fast thrombocytopenia is happening, how fast the blood pressures are changing, or proteinuria, you would be able to identify by that delta of the labs how it's changing. It will give you a very clear signal.

As we screen patients in transplant, we see those evolving very quickly and we can tell who's going to be in trouble. I think prospective screening is a really strong emphasis on those discussions, where to start, before you even go to significant drugs.

Dr. Goker-Alpan

Okay, thank you. This was very helpful actually for me. I am sure it was very helpful for the other attendees and who's going to be listening to this session later on. Obviously these are burning questions and unfortunately, gene therapy is very novel and we still actually continue as we go, learning about the potential issues.

So I thank everyone who's attending and I'm going to say stay tuned for the last session of this year, in December, that we're going to discuss the immune system and the immune system how it impacts LSDs and how LSDs impact the immune system. Thank you very much entirely.

Thank you very much.